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Public Comments on Developing an Unified Intercarrier Compensation:========

Title: Developing an Unified Intercarrier Compensation
FR Document Number: 2011-04399
Legacy Document ID:
RIN:
Publish Date: 3/2/2011 12:00:00 AM

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FCC 09-31 Notice of Inquiry GN Docket No. 09-51 - Broadband Plan for Our Future

EXHIBIT TABLE to accompany the Comment of The EMR Policy Institute June 7, 2009

No.	Name		City State	Info	TYPE
1	Litovitz, Theodore	PhD, Physics	Catholic University	Presentation used at Congressional Staff Briefing	
2				*Pathophysiology*, March 2009	Journal articles
3	Hillman, Donald	PhD Animal Science	East Lansing MI	Analysis of RF in home	Affidavit
4	Tully, Lisa	PhD Toxicology and Pharmacology	Boulder CO	Developing EHS test	Affidavit
5	Schou, John	PhD Agronomy Researcher	Cedar Falls IA	EHS symptoms wife had to move to WV	Affidavit
6	Schou, Diane	PhD Industrial Technology	Green Bank WV	Industrial Technology Severe EHS had to move to WV husband in IA	Affidavit
7	Bruno, William	PhD, Physics Researched at Los Alamos	Santa FE NM	Severe symptoms Comment in NAS record	Affidavit
8	Dauble, Janet	Non-profit organization	Frazier CA	MCS EHS support group founder increase in 10 yrs	Affidavit
9	Carney, Deborah	JD. BA-Human Biology	Golden CO	EMRPI VP CARE counsel Research subject	Affidavit
10	Fox, Nicols	Journalist	Renick WV	Documents severe EHS moved from ME to WV	Affidavit
11	Kleiber, Daniel	Farmer beekeeper	Waterloo WI	Type 1 diabetic documented insulin effects	Affidavit
12	Kleiber, Catherine	BA in biological science	Waterloo WI	Severe microwave sickness Dirty power and RF reactions Young children react as well	Affidavit
13	Savarin, Evelyn		Hampton NH	EHS from education exposure Documents with own meters	Affidavit

13B	Gherzi, Alex	Savarin's landlord	Hampton NH	Landlord to Savarin child can now sleep with WiFi off	Affidavit
14	Hurston, Ronald	M.D.	Wayland, MA	"It invites potentially tragic public health consequences."	Affidavit
15	Patton, Margaret	2-time cancer survivor	Wayland MA	Close to tower long legal battles to enforce zoning	Affidavit
16	Ide, Judith	Concerned citizen	Wayland, MA	Close to tower long legal battles to enforce bylaw	Affidavit
17	Lettieri, Linda	Liver cancer survivor	Fishkill NY	Had to leave job because cell tower was erected there	Affidavit
18	Pape, Beverly	Breast cancer	Dallas TX	Still in treatment for cancer EHS headaches cognition	Affidavit
19	Kayda, Valetta	2 brain tumors	Kelso WA	Tumor treatment caused EHS Moved 3 times already	Affidavit
20	Singer, Katie	EHS Reproductive health educator	Santa Fe, NM	Written 2 books on reproductive health has severe symptoms herself	Affidavit
21	DiGennaro JoTina	Substitute teacher Husband has prostate cancer	Bayville NY	Water tower antennas 50 ft from school deed covenant violated	Affidavit
22	Perrin, Madeleine	Mother of 2 young kids	Bayville NY	Can't get kids into another school tower 50ft away	Affidavit
23	Rollans, Marian & James	Farmers 39 years	Mt. Ulla NC	Fighting broadcast towers 3 cell towers close by EHS symptoms	Affidavit
24	Webster, Betsy	Concerned parent	Mt. Ulla NC	Fighting broadcast proposal 15 towers already nearby	
25	Davis, Ruth	EHS sufferer	Ouray CO	Notarized version to follow	Affidavit

26	Hinson, Katherine	Mother 15yr 13yr boys EHS	Plymouth VT	Left GA for boys' health	Affidavit
27	Russo, Kristin	Mother of 3 kids	Burlington MA	Water tower antenna at school Moved recently to avoid	Affidavit
28	Clark, Gayle	mother 14 yr old son	Sedgwick KS	WiFi at school and work Tower proposed near home	Affidavit
29	Hackett, Lucy	EHS Injury began in college	South Bend, IN	Difficulty finishing degree Antennas close to home and family now	Affidavit
30	Danner, Ruth		Juneau AK	2 WiMax towers proposed 4 co-locators proposed at church with daycare	Affidavit
31	Bubnis, Michelle	EHS neighbor's WiFi	Austin TX	many antennas One at church can no longer attend	Affidavit
32	Zack, Corina	Concerned citizen	Arlington Heights, IL	Antenna in church across the street from home	Affidavit
33	Reilly, Sarah	MCS EHS	Fairfax, CA	Has to move often 2003 WiFi brought it on	Affidavit
34	Frumberg, Maria	EHS Dr. Rea :letter	Plano, TX	Had to drop wireless TV access Letter from city shows no concern about WiFi	Affidavit
35	Ordogne, Kimberly	EHS	Plano, TX	Had to leave home Citywide WiFI No sympathy from city	Affidavit
36	Feudale, Elizabeth	MCS EHS Allergies immune problems	Allentown, PA	Cell towers nearby cannot tolerate home electronics	Affidavit
37	Olson, Veronica	Concerned parent	Plano, Texas	Concerned about citywide WiFi exposure to children	Affidavit
38	Hillman, Howard	Concerned citizen	Plano, Texas	Concerned about citywide WiFi exposure to children and immune-compromised people	Affidavit
39	Flynn, Angela	EHS came at job training near	Bethseda, MD	Moved to ease exposure EHS symptoms are Sleep muscle aches cognition	Affidavit

		antennas			
40	Lizik, Kyrie	EHS	Washington County WI	Smart meter aggravates Cannot use library – WiFi	Affidavit
41	Barris, Elizabeth	EHS documentary film maker	Santa Monica CA	Airport exposure an issue Must travel for work	Affidavit
42	Avola, JeanMarie	Concerned parent	Stoneham MA	Cell towers and WiFi in and near children's schools	Affidavit
43	Kelley, Elizabeth	Bioelectromagnetics Society member	Tucson ARIZ	Cell towers and WiFi in neighborhood Son's school has WiFi	Affidavit
44	Boca Raton, Florida	States of Colorado and Connecticut, Los Angeles County	Portland, Oregon Los Angeles public school district	**US states and municipalities are calling for revision of Section 704**	Proclamations and Resolutions
45	National Academies of Science	January 2008 Report		*Identification of Research Needs Relating to Potential Biological or Adverse Health Effects of Wireless Communication Devices* (NAS Report)	Research base inadequate for today's exposures
46	FDA nominates RF	To National Toxicology Program		Radiofrequency Radiation Emissions of Wireless Communication Devices	Research does not address typical RF exposures
47		NTP 2005 Fact Sheet on RF research	US federal government	Underscores inadequacy of research upon which US RF safety limits are based	
48	Carpenter, MD, Sage, Cindy	***The BioInitiative Report***	www.bioinitiative.org	*A Rationale for a Biologically-based Public Exposure Standard for Electromagnetic Fields (ELF and RF)*	On-line meta-analysis of EMR research
49 & 50	Carpenter and Sage	***Reviews in Environmental Health***	Peer-reviewed Scientific journal	*"Setting Prudent Health Policy for Electromagnetic Exposures"*	Journal article
51	Horst Eger, Klaus Uwe Hagen, Birgitt Lucas, Peter Vogel, and Helmut Voit	*Umwelt·Medizin·Gesell-schaft* 17,4 2004,	Research requested by German federal government	"The Influence of Being Physically Near to a Cell Phone Transmission Mast on the Incidence of Cancer"	Peer-reviewed Journal article
52	Carpenter	Amicus brief and	Review research	warn of the potential health consequences for	

	Olden Grigoriev Havas	statements on RF radiation and school children	and existing EMR safety limits	many students and staff if wireless technologies are deployed in their workplaces.	

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Pathophysiology xxx (2009) xxx–xxx

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Preface

There is an old joke with a well-known punch line about a man who has just fallen from the 86th floor of the Empire State Building in New York. As he passes the 30th floor, he is heard saying to himself 'so far, so good'...

Most of us laugh because we know where the man is headed, and that he must know too. But, our laughter usually has a guilty edge. We know that many of us are guilty of occasionally displaying a 'so far, so good' attitude in our own lives. We think of the smoker who says that about the possibility of getting lung cancer or heart disease and who counts on beating the odds because he feels healthy at the moment. That smoker will not find out if he won the bet until many years later, and by then it is often too late. The 'so far, so good' attitude to health is so common that people even kid themselves about it. One smoker told me that smoking would only cut a few years off his life, and that he did not mind losing the last few years because they are usually not much fun anyway.

Unlike the optimist in the joke, whose end is virtually certain, many of us live like the smoker, playing the odds and reassuring ourselves 'so far, so good'. Diseases like cancer usually take many years to develop, and we try not to think how some of the things we do casually can affect the long-term odds by compromising the natural processes that protect us. We rely on our bodies to be strong and resilient all the time. Yet, we know there are limits to the body's natural ability to reverse damage to cells. We also know that there may be gaps in the ability of our genetic endowment to cope with damage. At some level, we all know it is just common sense to try to minimize damage to our bodies and maximize the ability to repair.

These opening paragraphs provide a quick introduction to the theme of this issue of Pathophysiology and a summary of the point of view of its authors. The public is currently interested in possible hazards from radio frequency (RF) due to cellphones, towers, WiFi, etc. The concern is certainly warranted, but we are surrounded by electromagnetic fields (EMFs) of many frequencies, and there are also significant biological effects and known risks from low frequency EMF. The scientific problem is to determine the nature of EMF interaction with biological systems and develop ways of coping with harmful effects in all frequency ranges, as well as their cumulative effects. The practical problem is to minimize the harmful biological effects of all EMF.

The technical papers in this issue are devoted to an examination and an evaluation of evidence gathered by scientists regarding the effects of EMF, especially RF radiation, on living cells and on the health of human populations. The laboratory studies point to significant interactions of both power frequency and RF with cellular components, especially DNA. The epidemiological studies point to increased risk of developing certain cancers associated with long-term exposure to RF. Overall, the scientific evidence shows that the risk to health is significant, and that to deny it is like being in free-fall and thinking 'so far, so good'. We must recognize that there is a potential health problem, and that we must begin to deal with it responsibly as individuals and as a society.

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EMF effects on DNA

M. Blank and R. Goodman (USA): Electromagnetic Fields Stress Living Cells

Exhibit 2

Abbreviations: EMF, electromagnetic fields; Hz, hertz (cycles/s the unit of frequency); ELF, extremely low frequency (3–3 × 10^3 Hz) power frequency is 50–60 Hz; RF, radio frequency (band width 3 × 10^3 to 3 × 10^{11} Hz); UHF, ultrahigh frequency band the RF sub-division used for cell phones (3 × 10^8 to 3 × 10^9 Hz).

0928-4680/$ – see front matter © 2009 Published by Elsevier Ireland Ltd.
doi:10 1016/j.pathophys.2009.02.002

J.L. Phillips, N.P. Singh and H. Lai (USA): Electromagnetic Fields and DNA damage

H.W. Rűdiger (Austria): Genotoxic effects of electromagnetic exposure in vitro

EMF effects on the brain

H. Nittby, A. Brun, J. Eberhardt, L. Malmgren, B.R.R. Persson and L.G. Salford (Sweden): Increased blood–brain barrier permeability in mammalian brain seven days after exposure to the radiation from a GSM-900 mobile phone

L. Hardell, M. Carlberg and K Hansson Mild (Sweden): Epidemiological evidence for an association between use of wireless phones and tumor diseases

M. Kundi and H-P. Hutter (Austria): Mobile phone base stations – effects on wellbeing and health

L.L. Morgan: Estimating the risk of brain tumors from cellphone use: published case–control studies

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Z. Davanipour and E. Sobel: Long-term exposure to electromagnetic fields and the Risks of Alzheimer's disease and breast cancer: Further biological research

O. Johansson: Disturbance of the immune system by electromagnetic fields: A potentially underlying cause for cellular damage and tissue repair reduction which could lead to disease and impairment disturbance

A.F. Pourlis: Reproductive and developmental effects of EMF in vertebrate animal models

A. Balmori: Electromagnetic pollution from phone masts: Effects on wildlife

P. Huttunen, O. Hänninen and R.Myllylä: FM-radio and TV tower signals can cause spontaneous hand movements near moving RF reflector

C. Blackman: Cell Phone Radiation: Evidence from ELF and RF studies supporting more inclusive risk identification and assessment

Science as a guide to public policy

D. Gee: Late Lessons from early warnings: Towards realism and precaution with EMF?

C. Sage and D.O. Carpenter: Public Health Implications of Wireless Technologies

Special Issue on EMF

Bioelectromagnetics, the study of biological effects of electromagnetic fields (EMF), is an interdisciplinary science with a technical literature that is not easily accessible to the non-specialist. To increase access of the public to the technical literature and to the health implications of the scientific findings, the Bioinitiative Report was organized by an international group of scientists and published online at www.bioinitiative.org on August 31, 2007. The report has been widely read, and was cited in September 2008 by the European Parliament when it voted overwhelmingly that the current EMF safety standards were obsolete and needed to be reviewed.

This special issue of Pathophysiology includes scientific papers on the EMF issue by contributors to the Bioiniative Report, as well as others, and is prepared for scientists who are not specialists in bioelectromagnetics. Each paper is independent and self-contained. To help the reader appreciate how the different subjects contribute to an understanding of the EMF issue, the papers are arranged in groups that emphasize key areas, and the role of science in analyzing the problem and evaluating possible solutions. The subject headings are:

- DNA to show biological effects at the sub-cellular level that occur at very low EMF thresholds and across frequency ranges of the EM spectrum. Interactions with DNA may account for many of the effects of EMF, and they raise the possibility that genetic damage due to EMF can lead to cancer.
- The Brain is exposed to radiation from mobile phone antennas, and laboratory studies show that the radiation causes leakage of the protective blood–brain barrier, as well as the death of neurons in the brain. Radiation emitted from base stations can affect all who are in the vicinity. Epidemiological studies have shown a relation between exposure to mobile phones, base-stations and the development of brain tumors. Some epidemiological studies have significant flaws in design, and the risk of brain cancer may be greater than reported in the published results.
- In addition to the risk of brain cancer, EMF in the environment may contribute to diseases like Alzheimer's dementia and breast cancer in humans, as well as reproductive and developmental effects in animals in the wild. EMF affect the biochemical pathways and immunological mechanisms that link the different organ systems in our bodies and those of animals. The human body can act as an antenna for RF signals, and a small percentage of the population appears to be so sensitive to EMF that it interferes with their daily lives. In addition to the growing presence of EMF signals in the environment, the complexity of the signals may be important in altering biological responses. These are among the many factors that must be considered in approaching EMF safety issues.
- Science as a guide to public policy

Four centuries ago, when Francis Bacon envisioned a course for modern science, he expressed the idea that *knowledge is power that should be applied for the benefit of mankind.* It is in keeping with that ethical standard that the last two papers in this issue show how knowledge gained from scientific research can help solve problems arising from EMF in our environment. The first of these papers discusses the Precautionary Principle, its growing acceptance as a rational approach to environmental issues, and how past experience can help us deal with the EMF issue. The second paper, by the editors of the original BioInitiative Report, is an update on how best to deal with the challenge of EMF in the environ-

Please cite this article in press as: M. Blank, Preface, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.02.002

ment and, specifically, the problems accompanying wireless technologies.

We trust that the reviews and original research papers will increase awareness of the growing impact of EMF in the environment, and the need for modern society to deal expeditiously with the potential health problems brought to light by EMF research.

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22 January 2009

Please cite this article in press as: M. Blank, Preface, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.02.002

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Pathophysiology xxx (2009) xxx–xxx

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Electromagnetic fields stress living cells

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Received 30 January 2009; accepted 30 January 2009

Abstract

Electromagnetic fields (EMF), in both ELF (extremely low frequency) and radio frequency (RF) ranges, activate the cellular stress response, a protective mechanism that induces the expression of stress response genes, e.g., HSP70, and increased levels of stress proteins, e.g., hsp70. The 20 different stress protein families are evolutionarily conserved and act as 'chaperones' in the cell when they 'help' repair and refold damaged proteins and transport them across cell membranes. Induction of the stress response involves activation of DNA, and despite the large difference in energy between ELF and RF, the same cellular pathways respond in both frequency ranges. Specific DNA sequences on the promoter of the HSP70 stress gene are responsive to EMF, and studies with model biochemical systems suggest that EMF could interact directly with electrons in DNA. While low energy EMF interacts with DNA to induce the stress response, increasing EMF energy in the RF range can lead to breaks in DNA strands. It is clear that in order to protect living cells, EMF safety limits must be changed from the current thermal standard, based on energy, to one based on biological responses that occur long before the threshold for thermal changes.

Keywords: DNA; Biosynthesis; Electromagnetic fields; ELF; RF

1. Electromagnetic fields (EMF) alter protein synthesis

Until recently, genetic information stored in DNA was considered essentially invulnerable to change as it was passed on from parent to progeny. Mutations, such as those caused by cosmic radiation at the most energetic end of the EM spectrum, were thought to be relatively infrequent. The model of gene regulation was believed to be that the negatively charged DNA was tightly wrapped up in the nucleus with positively charged histones, and that most genes were 'turned off' most of the time. Of course, different regions of the DNA code are being read more or less all the time to replenish essential proteins that have broken down and those needed during cell division.

New insights into the structure and function of DNA have resulted from numerous, well-done laboratory studies. The demonstration that EMF induces gene expression and the synthesis of specific proteins [1,2] generated considerable controversy from power companies, government agencies, physicists, and most recently, cell phone companies. Physicists have insisted that the reported results were not possible because there was not enough energy in the power frequency range (ELF) to activate DNA. They were thinking solely of mechanical interaction with a large molecule and not of the large hydration energy tied up in protein and DNA structures that could be released by small changes in charge [3]. Of the biologists who accepted such results [4], most thought that the EMF interaction originated at, and was amplified by, the cell membrane and not with DNA.

It is now generally accepted that weak EMF in the power frequency range can activate DNA to synthesize proteins. An EMF reactive sequence in the DNA has been identified [5] and shown to be transferable to other gene promoters [6]. This DNA sequence acts as an EMF sensitive antenna

Abbreviations: EMF, electromagnetic fields; Hz, hertz; ELF, extremely low frequency; RF, radio frequency; MAPK, mitogen activated protein kinase; ERK1\2, extracellular signal regulated kinase; JNK, c-Jun-terminal kinase p38MAPK; SAPK, stress activated protein kinase; NADH, nicotinamide adenine dinucleotide dehydrogenase; ROS, reactive oxygen species.

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doi:10.1016/j.pathophys.2009.01.006

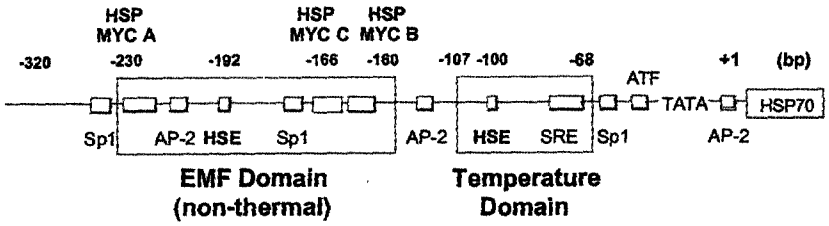

Fig. 1. Diagram of the HSP70 promoter showing the two different DNA sequences that have been identified as activated by EMF (non-thermal) and by thermal stimuli, respectively. The EMF domain contains three nCTCTn consensus sequences (electromagnetic response elements; EMRE), and differs from the consensus sequence (nGAAn) in the temperature or thermal domain.

that responds to EMF when transfected into reporter genes. Research at the more energetic levels of power frequency [7] and in the RF [8] ranges has shown that exposure to EMF can lead to breaks in the DNA strands. Therefore, DNA can no longer be considered unaffected by environmental EMF levels. It can be activated and damaged by EMF at levels that are considered safe [9]. The vulnerability of DNA to environmental influences and the possible dangers associated with EMF, had been underscored by discovery of EMF activation of the cellular stress response in the ELF range [10,11]. The cellular stress response is an unambiguous signal by the cell that EMF is potentially harmful.

2. Physiological stress and cellular stress

Discussions of physiological stress mechanisms usually describe responses of the body to pain, fear, 'oxygen debt' from muscle overexertion. These responses are mediated by organ systems. For example, the nervous system transmits action potentials along a network of nerves to cells, such as adrenal glands, that release rapidly acting agents such as epinephrine and norepinephrine and slower acting mineralocorticoids. These hormones are transported throughout the body by the circulatory system. They mobilize the defenses to cope with the adverse conditions and enable the body to 'fight or flee' from the noxious stimuli. The defensive actions include changes in heart rate, breathing rate, muscle activity, etc.

In addition to the responses of organ systems, there are protective mechanisms at the cellular level known as the cellular stress response. These mechanisms are activated by damage to cellular components such as DNA and protein [12], and the responses are characterized by increased levels of stress proteins [13] indicating that stress response genes have been upregulated in response to the stress.

The first stress response mechanism identified was the cellular reaction to sharp increases in temperature [14] and was referred to as 'heat shock', a term that is still retained in the nomenclature of the protective proteins, the hsps, heat shock proteins. Stress proteins are designated by the prefix 'hsp' followed by a number that gives the molecular weight in kilodaltons. There are about 20 different protein families ranging in molecular weight from a few kilodaltons to over 100 kD, with major groups of proteins around 30 kD, 70 kD and 90 kD.

Research on the 'heat shock' response has shown that hsp synthesis is activated by a variety of stresses that are potentially harmful to cells, including physical stimuli like pH and osmotic pressure changes, as well as chemicals such as alcohol and toxic metal ions like Cd^{2+}. EMF is a recent addition to the list of physical stimuli. It was initially shown in the power frequency (extremely low frequency, ELF) range [13], but shortly afterwards, radio frequency (RF) fields [15] and amplitude modulated RF fields [16] were shown to activate the same stress response.

Studies of stress protein stimulation by low frequency EMF have focused on a specific DNA sequence in the gene promoter that codes for hsp70, a major stress protein. Synthesis of this stress protein is initiated in a region of the promoter (see Fig. 1) where a transcription factor known as heat shock factor 1 (HSF-1) binds to a heat shock element (HSE). This EMF sensitive region on the HSP70 promoter is upstream from the thermal domain of the promoter and is not sensitive to increased temperature. The binding of HSF-1 to HSE occurs at −192 in the HSP70 promoter relative to the transcription initiation site. The EMF domain contains three nCTCTn myc-binding sites −230, −166 and −160 relative to the transcription initiation site and upstream of the binding sites for the heat shock (nGAAn) and serum responsive elements [5,6,17,18]. The electromagnetic response elements (EMREs) have also been identified on the c-*myc* promoter and are also responsive to EMF. The sensitivity of the DNA sequences, nCTCTn, to EMF exposures has been demonstrated by transfecting these sequences into CAT and Luciferase reporter genes [6]. Thus, the HSP70 promoter contains different DNA regions that are specifically sensitive to different stressors, thermal and non-thermal.

Induction of increased levels of the major stress protein, hsp70, by EMF is rapid, within 5 min. Also it occurs at extremely low levels of energy input, 14 orders of magnitude lower than with a thermal stimulus [10]. The far greater sensitivity to EMF than to temperature change in elevating the protective protein, hsp70, has been demonstrated to have potential clinical application, preventing injury from ischemia reperfusion [19–21]. George et al. [22] have shown the non-invasive use of EMF-induced stress proteins improved hemodynamic parameters during reperfusion

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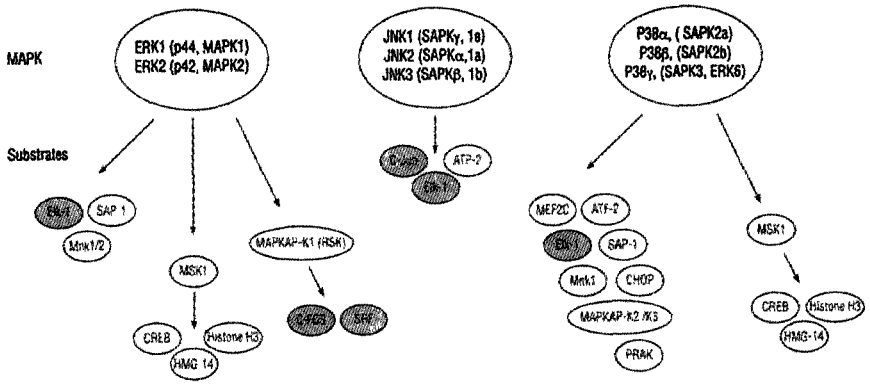

Fig. 2. The four mitogen activated protein kinase (MAPK) signaling cascades identified to date are: extracellular signal regulated kinase 1/2 (ERK), c-Jun-terminal kinase (JNK), p38MAPK and stress activated protein kinase (SAPK). Elements of the three MAPkinase pathways that have been identified as activated by EMF are shown as the shaded circles.

following ischemia. This effect occurred in the absence of measurable increased temperature.

3. EMF interaction with signaling pathways

EMF penetrate cells unattenuated and so can interact directly with the DNA in the cell nucleus, as well as other cell constituents. However, biological agents are impeded by membranes and require special mechanisms to gain access to the cell interior. Friedman et al. [23] have demonstrated that the initial step in transmitting extracellular information from the plasma membrane to the nucleus of the cell occurs when NADH oxidase rapidly generates reactive oxygen species (ROS). These ROS stimulate matrix metalloproteinases that allow them to cleave and release heparin binding epidermal growth factor. This secreted factor activates the epidermal growth receptor, which in turn activates the extracellular signal regulated kinase 1\2 (ERK) cascade. The ERK cascade is one of the four mitogen-activated protein kinase (MAPK) signaling cascades that regulate transcriptional activity in response to extracellular stimuli. The elements of the three MAPK signaling cascades implicated in exposures to ELF and RF are highlighted in Fig. 2.

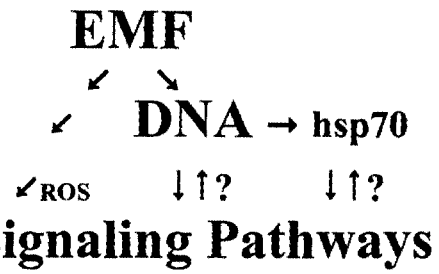

Fig. 3. The signaling pathways and the stress response are activated by EMF. The activation mechanisms discussed in the text are indicated by arrows. In the stress response, DNA activation leads to hsp synthesis and may be due to direct EMF interaction with DNA. The signaling pathways are activated by reactive oxygen species (ROS) that are probably generated by EMF. Possible interactions between the pathways, DNA and hsp are indicated with question marks. In any case, EMF leads to activation of all the processes shown.

The four MAPK cascades are: (1) ERK, (2) c-Jun-terminal kinase (JNK), (3) stress activated protein kinase (SAPK) and (4) p38SAPK. Each of the cascades is composed of three to six tiers of protein kinases, and their signals are transmitted by sequential phosphorylation and activation of the protein kinases in each of the tiers. The result is activation of a large number of regulatory proteins, which include a set of transcription factors, e.g., c-Jun, c-Fos, hsp27 and hsp70. Activation of the stress response is accompanied by activation of specific signal transduction cascades involved in regulating cell proliferation, differentiation and metabolism [24–26]. The MAPK pathways have been characterized in several cell types [24,27–30]. Exposure to non-thermal ELF as well as thermal RF affects the expression of many cellular proteins [23–25] (Fig. 3).

The elevated expression of these protein transcription factors participate in the induction of various cellular processes, including several that are affected by cell phones, e.g., replication and cell-cycle progression [25,31] and apoptosis [32]. RF fields have been shown to activate specific transcription factor binding that stimulate cell proliferation and induce stress proteins [25,33]. It has been reported [31] that within 10 min of cell phone exposures, two MAPKinase cascades, p38 and ERK1\2, are activated. Both ELF and RF activate the upregulation of the HSP70 gene and induction of elevated levels of the hsp70 protein. This effect on RNA transcription and protein stability is controlled by specific protein transcription factors that are elements of the mitogen MAPK cascade.

EMF also stimulate serum response factor which binds to the serum response element (SRE) through ERK MAPK activation and is associated with injury and repair *in vivo* and *in vitro*. The SRE site is on the promoter of an early response gene, c-fos, which under specific cellular circumstances has oncogenic properties. The c-fos promoter is EMF-sensitive; a 20 min exposure to 60 Hz 80mG fields significantly increases c-*fos* gene expression [34]. The SRE accessory protein,

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Elk-1, contains a growth-regulated transcriptional activation domain. ERK phosphorylation potentiates Elk-1 and transactivation at the c-*fos* SRE [29].

During the past twenty years, the growing use of cellular phones has aroused great concern regarding the health effects of exposure of the brain to 900 MHz RF waves. Despite claims that the energy level is too low to induce changes in DNA and that the devices are safe, the non-thermal effects that have been demonstrated at both ELF and RF exposure levels can cause physiological changes in cells and tissues even at the level of DNA. Finally, it should be mentioned that some of the pathways described in this section also have roles in protein synthesis via RNA polymerase III, an enzyme in oncogenic pathways [35] and could, therefore, provide a mechanistic link between cancer and EMF exposure.

4. Cells affected by the stress response

Reviews on EMF and the stress response have appeared for the ELF range [13] and for the RF range [36]. The most recent review was published online in section 7 of the Bioinitiative Report [9], and it summarized both ELF and RF studies, mainly at frequencies 50 Hz, 60 Hz, 900 MHz and 1.8 GHz. The citations in that review were not exhaustive, but the different frequencies and biological systems represent the diversity of results on stimulation of DNA and stress protein synthesis in many different cells. It is clear that the stress response does not occur in reaction to EMF in all types of cells, and sometimes because of the use of tissue cultured cell lines, even the same cell line can give opposite results in the same laboratory [37].

Many different types of cells have been shown to respond to EMF, both *in vivo* and *in vitro*, including epithelial, endothelial and epidermal cells, cardiac muscle cells, fibroblasts, yeast, *E. coli*, developing chick eggs, and dipteran cells (see Bioinitiative Report [9], section 7). Tissue cultured cells are less likely to show an effect of EMF, probably because immortalized cells have been changed significantly to enable them to live indefinitely in unnatural laboratory conditions. This may also be true of cancer cells, although some (e.g., MCF7 breast cancer cells) have responded to EMF [38,39], and in HL60 cells, one cell line responds to EMF while another does not [24]. Czyz et al. [16] found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not.

A broad study of genotoxic effects (i.e., DNA damage) in different kinds of cells [40] found no effects with lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Other studies [41,42] have also found that the blood elements, such as lymphocytes and monocytes are natural cells that have not responded. Since mobile cells can easily move away from a stress, there would be little selective advantage and evolutionary pressure for developing the stress response. The lack of response by skeletal muscle cells is related to the need to desensitize the cells to excessive heating during activity. Unlike slow muscle fibers that do synthesize hsp70, cells containing fast muscle fibers do not synthesize hsp70 to protect them from over-reacting to the high temperatures reached a during activity.

Table 1
Biological thresholds in the ELF range.

Biological system	Threshold (μT)[a]	Reference
Acceleration of reaction rates		
Na,K-ATPase	0.2–0.3	Blank and Soo [49]
cytochrome oxidase	0.5–0.6	Blank and Soo [43]
ornithine decarboxylase	~2	Mullins et al. [58]
malonic acid oxidation	<0.5	Blank and Soo [59]
Biosynthesis of stress proteins		
HL60, Sciara, yeast,	<0.8	Goodman et al. [11]
breast (HTB124, MCF7)	<0.8	Lin et al. [39]
chick embryo (anoxia)	~2	DiCarlo et al. [60]
Breast cancer (MCF7) cell growth block melatonin inhibition	0.2 < 1.2	Liburdy et al. [38]
Leukemia epidemiology	0.3–4	Ahlbom et al. [61] Greenland et al. [62]

[a] The estimated values are for departures from the baseline, although Mullins et al. (1999) and DiCarlo et al. (2000) generally give inflection points in the dose–response curves. The leukemia epidemiology values are not experimental and are listed for comparison.

5. EMF–DNA interaction mechanisms: electron transfer

The biochemical compounds in living cells are composed of charges and dipoles that can interact with electric and magnetic fields by various mechanisms. An example discussed earlier is the generation of reactive oxygen species (ROS) in activation of the ERK signaling cascade. The cellular stress response leading to the synthesis of stress proteins is also activated by EMF. However, the specific reaction is not known, except that it is stimulated by very weak EMF. For this reason, our focus has been on molecular processes that are most sensitive to EMF and that could cause the DNA to come apart to initiate biosynthesis. We have suggested that direct EMF interaction with electrons in DNA is likely for the following reasons:

- The largest effects of EMF would be expected on electrons because of their high charge to mass ratio. At the sub-atomic level, one assumes that electrons respond instantaneously compared to protons and heavier atomic nuclei, as in the Born-Oppenheimer Approximation. The very low field strengths and durations that activate the stress response and other reactions (Table 1) suggest interaction with electrons, and make ion-based mechanisms unlikely.
- Weak ELF fields have been shown to affect the rates of electron transfer reactions [43,44]. A 10 μT magnetic field exerts a very small force of only $\sim 10^{-20}$ N on a unit charge,

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but this force can move an isolated electron more than a bond length, ~1 nm, in ~1 nanosecond.
- There is a specific EMF responsive DNA sequence that is associated with the response to EMF (Fig. 1), and that retains this property when transfected
- Displacement of electrons in DNA would cause local charging that has been shown to lead to disaggregation of biopolymers [45].
- As the energy in an EMF stimulus increases, there is an increase in single strand breaks, followed by double strand breaks, suggesting an interaction with EMF at all energy levels [46].

Effects of EMF on electrons in chemical reactions were detected indirectly in studies on the Na,K-ATPase [47], a ubiquitous enzyme that establishes the normal Na and K ion gradients across cell membranes. Electric and magnetic fields, each accelerated the reaction only when the enzyme was relatively inactive. It is reasonable to assume that the threshold response occurs when the same charge is affected by the two fields, so the velocity (v) of the charge (q) could be calculated from these measurements and its nature determined. Assuming both fields exert the same force at the threshold, the electric (E) and the magnetic (B) forces should be equal.

$$F = qE = qvB. \quad (1)$$

From this $v = E/B$, the ratio of the threshold fields, and by substituting the measured thresholds [48,49], $E = 5 \times 10^{-4}$ V/m and $B = 5 \times 10^{-7}$T (0.5 μT), we obtain $v = 10^3 m/s$. This very rapid velocity, similar to that of electrons in DNA [50], indicated that electrons were probably involved in the ion transport mechanism of the Na,K-ATPase [47]. An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the ELF field has had a chance to change. This means that a low frequency sine wave signal is effectively a repeated DC pulse. This is true of all low frequency effects on fast moving electrons.

Studies of effects of EMF on electron transfer in cytochrome oxidase, ATP hydrolysis by the Na,K-ATPase, and the Belousov–Zhabotinski (BZ) redox reaction, have led to certain generalizations:

- EMF can accelerate reaction rates, including electron transfer rates
- EMF acts as a force that competes with the chemical forces in a reaction. The effect of EMF varies inversely with the intrinsic reaction rate, so EMF effects are only seen when intrinsic rates are low. (This is in keeping with the therapeutic efficacy of EMF on injured tissue, while there is usually little or no effect on normal tissue.)
- Experimentally determined thresholds are low (~0.5 μT) and comparable to levels found by epidemiology. See Table 1.
- Effects vary with frequency, with different optima for the reactions studied: The two enzymes showed broad frequency optima close to the reaction turnover numbers for Na,K-ATPase (60 Hz) and cytochrome oxidase (800 Hz), suggesting that EMF interacted optimally when in synchrony with the molecular kinetics. This is not true for EMF interactions with DNA, which are stimulated in both ELF and RF ranges and do not appear to involve electron transfer reactions with well-defined kinetics.

Probably the most convincing evidence for a frequency sensitive mechanism that involves stimulation of DNA is activation of protein synthesis in striated muscle. In this natural process, specific muscle proteins are synthesized by varying the rate of the (electrical) action potentials in the attached nerves [51]. The ionic currents of the action potentials that flow along and through the muscle membranes, also pass through the muscle cell nuclei that contain the DNA codes for the muscle proteins. Two frequencies were studied in muscle, high (100 Hz) and low (10 Hz) frequency, corresponding to the frequencies of the fast muscles and slow muscles that have different contraction rates and different muscle proteins. In the experiments, either the fast or slow muscle proteins were synthesized at the high or low frequency stimulation rates corresponding to the frequency of the action potentials. The clear dependence of the protein composition on the frequency of the action potentials indicates a relation between stimulation and activation of DNA in muscle physiology. The process is undoubtedly far more complicated and unlikely to be a simple electron transfer reaction as with cytochrome oxidase. It is more probable that an entire region of DNA coding for a group of related proteins is activated simultaneously.

A mechanism based on electron movement is in keeping with the mV/m electric field and μT magnetic field thresholds that affect the Na,K-ATPase. The very small force on a charge ($\sim 10^{-20}$ N) can affect an electron, but is unlikely to have a direct effect on much more massive ions and molecules, especially if they are hydrated. Ions are affected by the much larger DC electric fields of physiological membrane processes. The low EMF energy can move electrons, cause small changes in charge distribution and release the large hydration energy tied up in protein and DNA structures [3]. Electrons have been shown to move in DNA at great speed [50], and we have suggested that RF and ELF fields initiate the stress response by directly interacting and accelerating electrons moving within DNA [52,53].

A mechanism based on electron movement also provides insight into why the same stress response is stimulated by both ELF and RF even though the energies of the two stimuli differ by orders of magnitude. A typical ELF cycle at 10^2Hz lasts 10^{-2} s and a typical RF cycle at 10^{11} Hz lasts 10^{-11} s. Because the energy is spread over a different number of cycles/second in the two ranges, the energy/cycle is the same in both ELF and RF ranges. Since electron movement occurs much faster than the change of field, both frequencies are seen by rapidly moving electrons as essentially DC pulses. Each cycle contributes to electron movement at both

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frequencies, but more rapidly at the higher frequency. The fluctuation of protons between water molecules in solution at a frequency of about 10^{12} Hz [54] gives an indication of the speed of electron movement, and may suggest an upper limit of the frequency in which sine wave EMF act as DC pulses.

6. DNA biology and the EM spectrum

Research on DNA and the stress response has shown that the same biology occurs across divisions of the EM spectrum, and that EMF safety standards based on cellular measures of potential harm should be much stricter. These data also raise questions about the utility of spectrum sub-divisions as the basis for properly assessing biological effects and setting separate safety standards for the different sub-divisions. The frequencies of the EM spectrum form a continuum, and division into frequency bands is only a convenience that makes it easier to assign and regulate different portions of the spectrum for practical uses, such as the different design requirements of devices for EMF generation and measurement. Except for the special case of the visual range, the frequency bands are not based on biology, and the separate bands now appear to be a poor way of dealing with biological responses needed for evaluating safety. The DNA studies indicate the need for an EMF safety standard rooted in biology and a rational basis for assessing health implications.

DNA responses to EMF can be used to create a single scale for evaluation of EMF dose because:

- The same biological responses are stimulated in ELF and RF ranges.
- The intensity of EMF interactions with DNA leads to greater effects on DNA as the energy increases with frequency. In the ELF range, the DNA is only activated to initiate protein synthesis, while single and double strand breaks occur in the more energetic RF and ionizing ranges.

A scale based on DNA biology also makes possible an approach to a quantitative relation between EMF dose and disease. This can be done by utilizing the data banks that have been kept for A-bomb exposure and victims of nuclear accidents, data that link exposure to ionizing radiation and subsequent development of cancer. Utilizing experimental studies of DNA breaks with ionizing radiation, it is possible in principle to relate cancer incidence to EMF exposures. It should be possible to determine single and double strand breaks in a standard preparation of DNA, caused by exposure to EMF for a specified duration, under standard conditions. Although many studies of DNA damage and repair rates under different conditions would be needed, this appears to be a possible experimental approach to assessing the relation between EMF exposure and disease.

7. The stress response and safety standards

Most scientists believe that basic research eventually pays off in practical ways. This has certainly been true of EMF research on the stress response, where EMF stimulated stress proteins have been used to minimize damage to ischemic tissues on reperfusion. However, more importantly, biological effects stimulated by both ELF and RF have shown that the standards used for developing safety guidelines are not protective of cells.

First and foremost, it is important to realize that the stress response occurs in reaction to a potentially harmful environmental influence. The stress response is an unambiguous indication that cells react to EMF as potentially harmful. It is therefore an indication of compromised cell safety, given by the cell, in the language of the cell. The low threshold level of the stress response shows that the current safety standards are much too high to be considered safe.

In general, cellular processes are unusually sensitive to fields in the environment. The biological thresholds in the ELF range (Table 1) are in the range of 0.5–1.0 μT—not very much higher than the ELF backgrounds of ~0.1 μT. The relatively low field strengths that can affect biochemical reactions is a further indication that cells are able to sense potential danger long before there is an increase in temperature.

EMF research has also shown that exposure durations do not have to be prolonged to have an effect. Litovitz et al. [55,56], working with the enzyme ornithine decarboxylase, showed an EMF response when cells were exposed for only 10 s to ELF or ELF modulated 915 MHz, providing that the exposure was continuous. Gaps in the sine wave resulted in a reduced response, and interference with the sine wave in the form of superimposed ELF noise also reduced the response [57]. The interfering effect of noise has been shown in the RF range by Lai and Singh [46], who reported that noise interferes with the ability of an RF signal to cause breaks in DNA strands. The decreased effect when noise is added to a signal is yet another indication that EMF energy is not the critical factor in causing a response. In fact, EMF noise appears to offer a technology for mitigating potentially harmful effects of EMF in the environment.

EMF research has shown that the thermal standard used by agencies to measure safety is at best incomplete, and in reality not protective of potentially harmful non-thermal fields. Non-thermal ELF mechanisms are as effective as thermal RF mechanisms in stimulating the stress response and other protective mechanisms. The current safety standard based on thermal response is fundamentally flawed, and not protective.

Finally, since both ELF and RF activate the same biology, simultaneous exposure to both is probably additive and total EMF exposure is important. Safety standards must consider total EMF exposure and not separate standards for ELF and RF ranges.

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Please cite this article in press as: M. Blank, R. Goodman, Electromagnetic fields stress living cells, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.01.006

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Pathophysiology xxx (2009) xxx–xxx

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Electromagnetic fields and DNA damage

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Received 24 October 2008; received in revised form 16 November 2008; accepted 16 November 2008

Abstract

A major concern of the adverse effects of exposure to non-ionizing electromagnetic field (EMF) is cancer induction. Since the majority of cancers are initiated by damage to a cell's genome, studies have been carried out to investigate the effects of electromagnetic fields on DNA and chromosomal structure. Additionally, DNA damage can lead to changes in cellular functions and cell death. Single cell gel electrophoresis, also known as the 'comet assay', has been widely used in EMF research to determine DNA damage, reflected as single-strand breaks, double-strand breaks, and crosslinks. Studies have also been carried out to investigate chromosomal conformational changes and micronucleus formation in cells after exposure to EMF. This review describes the comet assay and its utility to qualitatively and quantitatively assess DNA damage, reviews studies that have investigated DNA strand breaks and other changes in DNA structure, and then discusses important lessons learned from our work in this area.

Keywords: Electromagnetic field; DNA damage; Comet assay; Radiofrequency radiation; Cellular telephone

1. The comet assay for measurement of DNA strand breaks

DNA is continuously damaged by endogenous and exogenous factors and then repaired by DNA repair enzymes. Any imbalance in damage and repair and mistakes in repair result in accumulation of DNA damage. Eventually, this will lead to cell death, aging, or cancer. There are several types of DNA lesions. The common ones that can be detected easily are DNA strand breaks and DNA crosslinks. Strand breaks in DNA are produced by endogenous factors, such as free radicals generated by mitochondrial respiration and metabolism, and by exogenous agents, including UV, ionizing and non-ionizing radiation, and chemicals.

There are two types of DNA strand breaks: single- and double-strand breaks. DNA single-strand breaks include frank breaks and alkali labile sites, such as base modification, deamination, depurination, and alkylation. These are the most commonly assessed lesions of DNA. DNA double-strand breaks are very critical for cells and usually they are lethal. DNA strand breaks have been correlated with cell death [1–5], aging [6–8] and cancer [9–13].

Several techniques have been developed to analyze single- and double-strand breaks. Most commonly used is microgel electrophoresis, also called the 'comet assay' or 'single cell gel electrophoresis'. This technique involves mixing cells with agarose, making microgels on a microscope slide, lysing cells in the microgels with salts and detergents, removing proteins from DNA by using proteinase K, unwinding/equilibrating and electrophoresing DNA (under highly alkaline condition for assessment of single-strand breaks or under neutral condition for assessment of DNA double-strand breaks), fixing the DNA, visualizing the DNA with a fluorescent dye, and then analyzing migration patterns of DNA from individual cells with an image analysis system.

The comet assay is a very sensitive method of detecting single- and double-strand breaks if specific criteria are met. Critical criteria include the following. Cells from tissue culture or laboratory animals should be handled with care to minimize DNA damage, for instance, by avoiding light and high temperature. When working with animals exposed to EMF *in vivo*, it is better to anesthetize the animals with CO_2 before harvesting tissues for assay. Antioxidants

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doi:10.1016/j.pathophys.2008.11.005

such as albumin and sucrose, or spin-trap molecules such as α-phenyl-*tert*-butyl nitrone (PBN), should be added during dispersion of tissues into single cells. Cells should be lysed at 0–4 °C to minimize DNA damage by endonucleases. Additionally, antioxidants such as tris and glutathione, and chelators such as EDTA, should be used in the lysing solution. High concentrations of dimethylsulfoxide (DMSO) should be avoided due to its chromatin condensing effect. Treatment with proteinase K (PK; lyophilized DNAse-free proteinase-K from Amresco is ideal) at a concentration of 0.5–1 mg/ml (depending upon cell type and number of cells in the microgel) should be used for 1–2 h at 37 °C to reveal all possible strand breaks which otherwise may go undetected due to DNA–protein crosslinks. Longer times in PK will lead to loss of smaller pieces of DNA by diffusion. Glass slides should be chosen based on which high resolution agarose (3:1 high resolution agarose from Amresco is ideal) will stick well to the slide and on the ability of the specimen to be visualized without excessive fluorescence background. Choice of an electrophoresis unit is important to minimize slide-to-slide variation in DNA migration pattern. A unit with uniform electric field and buffer recirculation should be used. Electrophoresis buffers should have antioxidants and chelators such as DMSO and EDTA. DNA diffusion should be minimized during the neutralization step by rapidly precipitating the DNA. Staining should employ a sensitive fluorescent dye, such as the intercalating fluorescent labeling dye YOYO-1. A cell-selection criteria for analysis should be set before the experiment, such as not analyzing cells with too much damage, although, the number of such cells should be recorded.

There are different versions of the comet assay that have been modified to meet the needs of specific applications and to improve sensitivity. Using the most basic form of the assay, one should be able to detect DNA strand breaks in human lymphocytes that were induced by 5 rad of gamma-ray [14,15].

2. Radiofrequency radiation (RFR) and DNA damage

In a series of publications, Lai and Singh [16–19] reported increases in single- and double-strand DNA breaks, as measured by the comet assay, in brain cells of rats exposed for 2 h to a 2450-MHz RFR at whole body specific absorption rate (SAR) between 0.6 and 1.2 W/kg. The effects were blocked by antioxidants, which suggested involvement of free radicals. At the same time, Sarkar et al. [20] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150, and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals. Their data also suggested breakage of DNA strands after RFR exposure. Phillips et al. [21] were the first to study the effects of two forms of cell cellular phone signals, known as TDMA and iDEN, on DNA damage in Molt-4 human lymphoblastoid cells using the comet assay. These cells were exposed to relatively low intensities of the fields (2.4–26 μW/g) for 2–21 h. They reported both increased and decreased DNA damage, depending on the type of signal studied, as well as the intensity and duration of exposure. They speculated that the fields may affect DNA repair in cells. Subsequently, different groups of researchers have also reported DNA damage in various types of cells after exposure to cell phone frequency fields. Diem et al. [22] exposed human fibroblasts and rat granulosa cells to cell phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; for 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h of exposure to different cell phone modulations in both cell types. The intermittent exposure schedule caused a significantly stronger effect than continuous exposure. Gandhi and Anita [23] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users. Markova et al. [24] reported that GSM signals affected chromatin conformation and γ-H2AX foci that co-localized in distinct foci with DNA double-strand breaks in human lymphocytes. The effect was found to be dependent on carrier frequency. Nikolova et al. [25] reported a low and transient increase in DNA double-strand breaks in mouse embryonic stem cells after acute exposure to a 1.7-GHz field. Lixia et al. [26] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 h of exposure to a 1.8-GHz field at 3 W/kg. Sun et al. [27] reported an increase in DNA single-strand breaks in human lens epithelial cells after 2 h of exposure to a 1.8-GHz field at SARs of 3 and 4 W/kg. DNA damage caused by the field at 4 W/kg was irreversible. Zhang et al. [28] reported that an 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 h of exposure. Aitken et al. [29] exposed mice to a 900-MHz RFR at a SAR of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as by alkaline and pulsed-field gel electrophoresis. Gel electrophoresis revealed no significant change in single- or double-strand breaks in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome and the nuclear β-globin locus. Changes in sperm cell genome after exposure to 2450-MHz microwaves have also been reported previously by Sarkar et al. [20]. Related to this are several publications that have reported decreased motility and changes in morphology in isolated sperm cells exposed to cell phone radiation [30], sperm cells from animals exposed to cell phone radiation [31], and cell phone users [32–34]. Some of these *in vivo* effects could be caused by hormonal changes [35,36].

There also are studies reporting no significant effect of cell phone RFR exposure on DNA damage. After RFR-induced DNA damage was reported by Lai and Singh [16] using 2450-MHz microwaves and after the report of Phillips et al. [21] on cell phone radiation was published, Motorola funded a series of studies by Roti Roti and colleagues [37] at

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Washington University to investigate DNA strand breaks in cells and animals exposed to RFR. None of the studies reported by this group found significant effects of RFR exposure on DNA damage [38–40]. However, a different version of the comet assay was used in these studies. More recently, four additional studies from the Roti-Roti laboratories also reported no significant effects on DNA damage in cells exposed to RFR. Li et al. [41] reported no significant change in DNA strand breaks in murine C3H10T1/2 fibroblasts after 2 h of exposure to 847.74- and 835.02-MHz fields at 3–5 W/kg. Hook et al. [42] showed that a 24-h exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA-modulated RFR did not significantly alter the level of DNA damage. Lagroye et al. [43,44] also reported no significant change in DNA strand breaks, protein–DNA crosslinks, and DNA–DNA crosslinks in cells exposed to 2450-MHz RFR.

From other laboratories, Vijayalaxmi et al. [45] reported no increase in DNA stand breaks in human lymphocytes exposed *in vitro* to 2450-MHz RFR at 2.135 W/kg for 2 h. Tice et al. [46] measured DNA single-strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed for 3 or 24 h at average SARs of 1.0–10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes. McNamee et al. [47–49] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 h to a 1.9-GHz field at SAR up to 10 W/kg. Zeni et al. [50] reported that a 2-h exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes. Sakuma et al. [51] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to cell phone radiation for 2 and 24 h. No significant changes in DNA strand breaks were observed up to a SAR of 800 mW/kg. Stronati et al. [52] showed that 24 h of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells. Verschaeve et al. [53] reported that long-term exposure (2 h/day, 5 days/week for 2 years) of rats to 900-MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

3. Extremely low frequency electromagnetic fields (ELF EMF) and DNA damage

To complete the picture, a few words on the effects of ELF EMF are required, since cell phones also emit these fields and they are another common form of non-ionizing EMF in our environment. Quite a number of studies have indicated that exposure to ELF EMF could lead to DNA damage [54–69]. In addition, two studies [70,71] have reported effects of ELF fields on DNA repair mechanisms. Free radicals and interaction with transitional metals (e.g., iron) [60,62,63,69] have also been implicated to play a role in the genotoxic effects observed after exposure to these fields.

4. Some considerations on the effects of EMF on DNA

From this brief literature survey, no consistent pattern of RFR exposure inducing changes in or damage to DNA in cells and organisms emerges. However, one can conclude that under certain conditions of exposure, RFR is genotoxic. Data available are mainly applicable only to radiation exposure that would be typical during cell phone use. Other than the study of Phillips et al. [21], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

Differences in experimental outcomes are expected since many factors could influence the outcome of experiments in EMF research. Any effect of EMF has to depend on the energy absorbed by a biological organism and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these factors have been discussed in a talk given by one us (HL) in Vienna, Austria in 1998 [72].

Radiation from cell phone transmission has very complex patterns, and signals vary with the type of transmission. Moreover, the technology is constantly changing. Research results from one types of transmission pattern may not be applicable to other types. Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [21], which demonstrated that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks after exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used poor exposure procedures with very limited documentation of exposure parameters, e.g., using an actual cell phone to expose cells and animals, thus rendering the data from these experiments as questionable.

Another source of influence on experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [73] and organisms [74,75] may not all respond similarly to EMF.

Comment about the comet assay also is required, since it was used in many of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay could be misleading. Another concern is that most of the comet assay studies were carried out by experimenters who had no prior experience with this technique and mistakes

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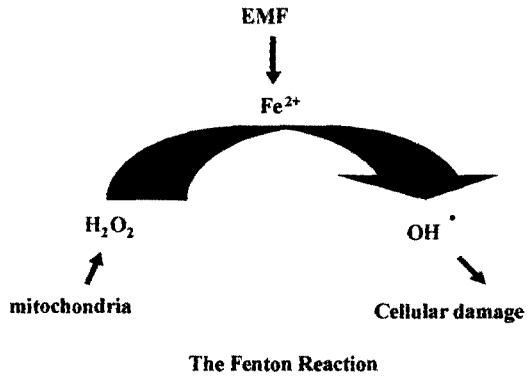

Fig. 1. A representation of the Fenton reaction and its role as a mediator in EMF-induced bioeffects.

were made. For example, in the study by Lagroye et al. [43] to investigate the effect of PK digestion on DNA migration after RFR exposure, PK was added to a lysing solution containing the detergent Triton X-100, which would inactivate the enzyme. Our experience indicates that the comet assay is a very sensitive and requires great care to perform. Thus, different detection sensitivities could result in different laboratories, even if the same procedures are followed. One way to solve this problem of experimental variation is for each research team to report the sensitivity of their comet assay, e.g., the threshold of detecting strand breaks in human lymphocytes exposed to X-rays. This information has generally not been provided for EMF-genotoxicity studies. Interestingly, when such information was provided, a large range of sensitivities have been reported. Malyapa et al. [40] reported a detection level of 0.6 cGy of gamma radiation in human lymphocytes, whereas McNamee et al. [76] reported 10–50 cGy of X-irradiation in lymphocytes, which is much higher than the generally acceptable detection level of the comet assay [15].

A drawback in the interpretation and understanding of experimental data from bioelectromagnetics research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which EMF produces changes in DNA is unknown. Since the energy level associated with EMF exposure is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced biochemical changes in cells.

One possibility is that DNA is damaged by free radicals that are formed inside cells. Free radicals affect cells by damaging macromolecules, such as DNA, protein, and membrane lipids. Several reports have indicated that EMF enhances free radical activity in cells [18,19,61,62,77,78], particularly via the Fenton reaction [62]. The Fenton reaction is a process catalyzed by iron in which hydrogen peroxide, a product of oxidative respiration in the mitochondria, is converted into hydroxyl free radicals, which are very potent and cytotoxic molecules (Fig. 1).

It is interesting that ELF EMF has also been shown to cause DNA damage. Furthermore, free radicals have been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR. Effects via the Fenton reaction predict how a cell would respond to EMF. For instance:

(1) Cells that are metabolically active would be more susceptible to EMF, because more hydrogen peroxide is generated by mitochondria to fuel the reaction.
(2) Cells that have high level of intracellular free iron would be more vulnerable to EMF. Cancer cells and cells undergoing abnormal proliferation have higher concentrations of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF. Consequently, this suggests that EMF could potentially be used for the treatment of cancer and hyperplastic diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells. One consequence of this consideration is that epidemiological studies of cancer incidence in cell phone users may not show a risk at all or even a protection effect.
(3) Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high levels of iron. Special molecular pumps are present on nerve cell nuclear membranes to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capacity for DNA repair, and DNA breaks could easily accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues, particularly in the brain. These particles could enhance free radical activity in cells and thus increase the cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, the more likely consequences of DNA damage in nerve cells include changes in cellular functions and in cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double-strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cell, the glial cell, can become cancerous as a result of DNA damage. The question is whether the damaged cells

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would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors—an increase, decrease, or no significant change in cancer risk could result from EMF exposure.

(4) On the other hand, cells with high amounts of antioxidants and antioxidative enzymes would be less susceptible to EMF. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

Additionally, the work of Blank and Soo [79] and Blank and Goodman [80] support the possibility that EMF exposure at low levels has a direct effect on electron transfer processes. Although the authors do not discuss their work in the context of EMF-induced DNA damage, the possibility exists that EMF exposure could produce oxidative damage to DNA.

5. Lessons learned

Whether or not EMF causes biological effects, let alone effects that are detrimental to human health and development, is a contentious issue. The literature in this area abounds with apparently contradictory studies, and as presented in this review, the literature specific to the effects of RFR exposure on DNA damage and repair in various biological systems is no exception. As a consequence of this controversy, there are several key issues that must be addressed—contrary data, weight of evidence, and data interpretation consistent with known science.

Consider that EMF does not share the familiar and comforting physical properties of chemical agents. EMF cannot be seen, tasted, smelled, or felt (except at high intensities). It is relevant, therefore, to ask, in what ways do scientists respond to data, especially if that data are contrary to their scientific beliefs or inconsistent with long-held hypotheses? Often such data are ignored, simply because it contradict what is accepted as conventional wisdom. Careful evaluation and interpretation of data may be difficult, because technologies used to expose biological systems to EMF and methodologies used to assess dosimetry generally are outside the experience of most biomedical scientists. Additionally, it is often difficult to assess differences in methodologies between studies, one or more of which were intended to replicate an original investigation. For instance, Malyapa et al. [40] reported what they claimed to be a replication of the work of Lai and Singh [16]. There were, however, significant differences in the comet analyses used by each group. Lai and Singh precipitated DNA in agarose so that low levels of DNA damage could be detected. Malyapa et al. did not. Lai and Singh treated their samples with PK to digest proteins bound to DNA, thus allowing DNA to move toward the positive pole during electrophoresis (unlike DNA, most proteins are negatively charged, and if they are not removed they will drag the DNA toward the negative pole). The Malyapa et al. study did not use PK. There were other methodological differences as well. Such is also the case in the study of Hook et al. [42], which attempted to replicate the work of Phillips et al. [21]. The latter group used a PK treatment in their comet assay, while the former group did not.

While credibility is enhanced when one can relate data to personal knowledge and scientific beliefs, it has not yet been determined how RFR couples with biological systems or by what mechanisms effects are produced. Even carefully designed and well executed RFR exposure studies may be summarily dismissed as methodologically unsound, or the data may be interpreted as invalid because of inconsistencies with what one believes to be correct. The quintessential example is the belief that exposure to RFR can produce no effects that are not related to the ability of RFR to produce heat, that is, to raise the temperature of biological systems [81,82]. Nonetheless, there are many examples of biological effects resulting from low-level (athermal) RFR exposure [83,84]. Consider here the work of Mashevich et al. [85]. This group exposed human peripheral blood lymphocytes to an 830-MHz signal for 72 h and at different average SARs (SAR, 1.6–8.8 W/kg). Temperatures ranged from 34.5 to 38.5 °C. This group observed an increase in chromosome 17 aneuploidy that varied linearly with SAR. Temperature elevation alone in the range of 34.5–38.5 °C did not produce this genotoxic effect, although significant aneuploidy was observed at higher temperatures of 40–41 °C. The authors conclude that the genotoxic effect of the radiofrequency signal used is elicited through a non-thermal pathway.

Also consider one aspect of the work of Phillips et al. [21]. In that study, DNA damage was found to vary in direction; that is, under some conditions of signal characteristics, signal intensity, and time of exposure, DNA damage increased as compared with concurrent unexposed controls, while under other conditions DNA damage decreased as compared with controls. The dual nature of Phillips et al.'s [21] results will be discussed later. For now consider the relationship of these results to other investigations. Adey et al. [86] performed an *in vivo* study to determine if rats treated *in utero* with the carcinogen ethylnitrosourea (ENU) and exposed to an 836.55-MHz field with North American Digital Cellular modulation (referred to as a TDMA field) would develop increased numbers of central system tumors. This group reported that rather than seeing an increase in tumor incidence in RFR-exposed rats, there was instead a decrease in tumor incidence. Moreover, rats that received no ENU but which were exposed to the TDMA signal also showed a decrease in the number of spontaneous tumors as compared

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with animals exposed to neither ENU nor the TDMA signal. This group postulated that their results may be mechanistically similar to the work of another group. Stammberger et al. [87] had previously reported that rats treated *in utero* with ENU and then exposed to low doses of X-irradiation exhibited significantly reduced incidences of brain tumors in adult life. Stammberger and colleagues [87] hypothesized that low-level X-irradiation produced DNA damage that then induced the repair enzyme 0^6-alkylguanine-DNA alkyltransferase (AT). Numerous groups have since reported that X-irradiation does indeed induce AT activity (e.g., [88,89]). In this context, it is significant that Phillips et al. [21] found that cells exposed *in vitro* to a TDMA signal identical to that used in the study of Adey et al. [86] produced a decrease in DNA damage under specific conditions of intensity and time of exposure (lower intensity, longer time; higher intensity, shorter time). These results raise the intriguing possibility that the decrease in tumor incidence in the study of Adey et al. [86] and the decrease in DNA damage in the study of Phillips et al. [21] both may have been the result of induction of AT activity resulting from DNA damage produced by exposure to the TDMA signal. This remains to be investigated.

Because the issue of RFR-induced bioeffects is contentious, and because the issue is tried in courtrooms and various public forums, a term heard frequently is weight of evidence. This term generally is used to describe a method by which all scientific evidence related to a causal hypothesis is considered and evaluated. This process is used extensively in matters of regulation, policy, and the law, and it provides a means of weighing results across different modalities of evidence. When considering the effects of RFR exposure on DNA damage and repair, modalities of evidence include studies of cells and tissues from laboratory animals exposed *in vivo* to RFR, studies of cells from humans exposed to RFR *in vivo*, and studies of cells exposed *in vitro* to RFR. While weight of evidence is gaining favor with regulators [90], its application by scientists to decide matters of science is often of questionable value. One of the reasons for this is that there generally is no discussion or characterization of what weight of evidence actually means in the context in which it is used. Additionally, the distinction between weight of evidence and strength of evidence often is lacking or not defined, and differences in methodologies between investigators are not considered. Consequently, weight of evidence generally amounts to what Krimsky [90] refers to as a "seat-of-the-pants qualitative assessment." Krimsky points out that according to this view, weight of evidence is "a vague term that scientists use when they apply implicit, qualitative, and/or subjective criteria to evaluate a body of evidence." Such is the case in the reviews by Juutilainen and Lang [91] and Verschaeve and Maes [92]. There is little emphasis on a critical analysis of similarities and differences in biological systems used, exposure regimens, data produced, and investigator's interpretations and conclusions. Rather, there is greater emphasis on the number of publications either finding or not finding an effect of RFR exposure on some endpoint. To some investigators, weight of evidence does indeed refer to the balance (or imbalance) between the number of studies producing apparently opposing results, without regard to critical experimental variables. While understanding the role these variables play in determining experimental outcome could provide remarkable insights into defining mechanisms by which RFR produced biological effects, few seem interested in or willing to delve deeply into the science.

A final lesson can be derived from a statement made by Gos et al. [93] referring to the work of Phillips et al. [21]. Gos and colleagues state, "The results in the latter study (Phillips et al., 1998) are puzzling and difficult to interpret, as no consistent increase or decrease in signal in the comet assay at various SARs or times of exposure was identified." This statement is pointed out because studies of the biological effects of exposure to electromagnetic fields at any frequency are often viewed as outside of or distinct from what many refer to as *mainstream science*. However, what has been perceived as an inconsistent effect is indeed consistent with the observations of bimodal effects reported in hundreds of peer-reviewed publications. These bimodal effects may be dependent on concentration of an agent, time of incubation with an agent, or some other parameter relating to the state of the system under investigation. For instance, treatment of B cells for a short time (30 min) with the protein kinase C activator phorbol 12,13-dibutyrate increased proliferative responses to anti-immunoglobulin antibody, whereas treatment for a longer period of time ($\geq$3 h) suppressed proliferation [94]. In a study of κ-opioid agonists on locomotor activity in mice, Kuzmin et al. [95] reported that higher, analgesic doses of κ-agonists reduced rearing, motility, and locomotion in non-habituated mice. In contrast, lower, subanalgesic doses increased motor activity in a time-dependent manner. Dierov et al. [96] observed a bimodal effect of all-trans-retinoic acid (RA) on cell cycle progression in lymphoid cells that was temporally related to the length of exposure to RA. A final example is found in the work of Rosenstein et al. [97]. This group found that the activity of melatonin on depolarization-induced calcium influx by hypothalamic synaptosomes from rats sacrificed late evening (2000 h) depended on melatonin preincubation time. A short preincubation time (10 min) stimulated uptake, while a longer preincubation (30 min) inhibited calcium uptake. These effects were also dependent on the time of day when the rats were sacrificed. Effects were maximal at 2000 h, minimal at 2400 h, and intermediate at 400 h. At 1000 h, only inhibitory effects of melatonin on calcium uptake were observed. These examples point out that what appears to be inconsistency may instead be real events related to and determined by the agents involved and the state of the biological system under investigation. The results of Phillips et al. [21] may be the result of signal modulation, signal intensity, time of exposure, or state of the cells. The results may indicate a bimodal effect, or they may, as the investigators suggest, represent time- and signal-dependant changes in the balance between damage and repair because of direct or indirect effects of RFR exposure on repair mechanisms.

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6. Summary

Exposure of laboratory animals *in vivo* and of cultured cells *in vitro* to various radiofrequency signals has produced changes in DNA damage in some investigations and not in others. That many of the studies on both sides of this issue have been done well is encouraging from a scientific perspective. RFR exposure does indeed appear to affect DNA damage and repair, and the total body of available data contains clues as to conditions producing effects and methodologies to detect them. This view is in contrast to that of those who believe that studies unable to replicate the work of others are more credible than the original studies, that studies showing no effects cancel studies showing an effect, or that studies showing effects are not credible simply because we do not understand how those effects might occur. Some may be tempted to apply incorrectly the teachings of Sir Karl Popper, one of the great science philosophers of the 20th century. Popper proposed that many examples may lend support to an hypothesis, while only one negative instance is required to refute it [98]. While this holds most strongly for logical subjects, such as mathematics, it does not hold well for more complex biological phenomena that are influenced by stochastic factors. Each study to investigate RFR-induced DNA damage must be evaluated on its own merits, and then studies that both show effects and do not show effects must be carefully evaluated to define the relationship of experimental variables to experimental outcomes and to assess the value of experimental methodologies to detect and measure these outcomes (see Section 2).

The lack of a causal or proven mechanism(s) to explain RFR-induced effects on DNA damage and repair does not decrease the credibility of studies in the scientific literature that report effects of RFR exposure, because there are several plausible mechanisms of action that can account for the observed effects. The relationship between cigarette smoking and lung cancer was accepted long before a mechanism was established. This, however, occurred on the strength of epidemiologic data [99]. Fortunately, relevant epidemiologic data relating long-term cell phone use (>10 years) to central nervous system tumors are beginning to appear [84,100–102], and these data point to an increased risk of acoustic neuroma, glioma and parotid gland tumors.

One plausible mechanism for RFR-induced DNA damage is free radical damage. After finding that two free radical scavengers (melatonin and N-tert-butyl-α-phenylnitrone) prevent RFR-induced DNA damage in rat brain cells, Lai and Singh [62] hypothesized that this damage resulted from free radical generation. Subsequently, other reports appeared that also suggested free radical formation as a result of RFR exposure [103–105]. Additionally, some investigators have reported that non-thermal exposure to RFR alters protein structure and function [106–109]. Scientists are familiar with molecules interacting with proteins through lock-and-key or induced-fit mechanisms. It is accepted that such interactions provide energy to change protein conformation and protein function. Indeed, discussions of these principles are presented in introductory biology and biochemistry courses. Perhaps then it is possible that RFR exposure, in a manner similar to that of chemical agents, provides sufficient energy to alter the structure of proteins involved in DNA repair mechanisms to the extent that their function also is changed. This has not yet been investigated.

When scientists maintain their beliefs in the face of contrary data, two diametrically opposed situations may result. On the one hand, data are seen as either right or wrong and there is no discussion to resolve disparities. On the other hand, and as Francis Crick [110] has pointed out, scientists who hold theoretically opposed positions may engage in fruitful debate to enhance understanding of underlying principles and advance science in general. While the latter certainly is preferable, there are external factors involving economics and politics that keep this from happening. It is time to acknowledge this and embark on the path of fruitful discussion. Great scientific discoveries await.

Acknowledgment

We thank Khushbu Komal and Ji-Sun Park for assistance in the preparation of the manuscript.

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Please cite this article in press as: J.L. Phillips, et al., Electromagnetic fields and DNA damage, Pathophysiology (2009), doi:10.1016/j.pathophys.2008.11.005

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Genotoxic effects of radiofrequency electromagnetic fields

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Received 24 October 2008; received in revised form 16 November 2008; accepted 16 November 2008

Abstract

101 publications are exploited which have studied genotoxicity of radiofrequency electromagnetic fields (RF-EMF) *in vivo* and *in vitro*. Of these 49 report a genotoxic effect and 42 do not. In addition, 8 studies failed to detect an influence on the genetic material, but showed that RF-EMF enhanced the genotoxic action of other chemical or physical agents. The controversial results may in part be explained by the different cellular systems. Moreover, inconsistencies may depend from the variety of analytical methods being used, which differ considerably with respect to sensitivity and specificity. Taking altogether there is ample evidence that RF-EMF can alter the genetic material of exposed cells *in vivo* and *in vitro* and in more than one way. This genotoxic action may be mediated by microthermal effects in cellular structures, formation of free radicals, or an interaction with DNA-repair mechanisms.

Keywords: Gene mutations; Cytogenetic effects; DNA fragmentation; Mechanisms of genotoxicity

1. Introduction

Alterations of genetic information in somatic cells are the key event in the process of carcinogenesis [1,2]. Consequently any agent, which has a genotoxic attribute is suspected also to be cancerogenic. This is the driving force behind the multitude of studies on genotoxicity of radiofrequency electromagnetic fields (RF-EMF), conducted so far. A total of 101 publications on genotoxicity studies of RF-EMF are exploited here, of which 49 report genotoxic effects, subsequently marked as GT(+) (Table 1), 43 do not (Table 2), and 9 find, that RF-EMF do not induce genotoxic events by itself but enhance the genotoxic action of other physical or chemical agents (Table 3). Thus, in contrast to several reviews in the past [3–6], it now became evident that non-thermal genotoxic effects of RF-EMF is convincingly demonstrated by a substantial number of published studies. The studies have been performed with a variety of different test systems – some studies used more than one test system – which will be assigned here to the three principle endpoints of a genotoxic action: (1) effect on chromosomes, (2) DNA fragmentation, and (3) gene mutations.

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doi:10.1016/j.pathophys.2008.11.004

2. Effect on chromosomes

This group comprises the analysis of numerical or structural anomalies of metaphase chromosomes (CA), sister-chromatid-exchanges (SCEs), and formation of micronuclei (MN). Of the 21 studies using CA, 9 are CA-positive, 11 CA-negative, and 1 reports an RF-induced enhancement of genotoxicity by X-rays. In general proliferating cells are required for the study of chromosomal effects, however, micronuclei have also been analysed in polychromatic erythrocytes and in exfoliated cells, for instance from buccal smears [7,8]. Moreover, aneuploidy rates of distinct chromosomes as well as chromosomal translocations can also be studied in interphase nuclei using fluorescence in situ hybridization (FISH). While structural aberrations detected by conventional CA are mainly lethal to the cell, translocations are persistent and may be passed to the cellular progeny. Using FISH increased levels of aneuploidy of chromosome 1, 10, 11, and 17 have been reported in human blood lymphocytes after RF-EMF exposure [9]. In metaphase chromosomes FISH may increase the sensitivity of chromosomal analysis [10] but this has only once been used for RF-EMF studies [11].

CA brings about to detect a variety of chromosomal aberrations. In contrast, micronuclei originate only from acentric

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Table 1
Publications which report RF-EMF related genotoxic effects.

Reference	Biological system	Genotoxic endpoint	Results and comments
Aitken et al. [45]	Mouse sperm	QPCR and comet assay	Gel electrophoresis revealed no gross evidence of increased single- or double-DNA strand breakage in spermatozoa. However, a detailed analysis of DNA integrity using QPCR revealed damage to both the mitochondrial genome ($p < 0.05$) and the nuclear-globin locus ($p < 0.01$).
Balode [46]	Cow erythrocytes	Micronuclei (MN)	The counting of micronuclei in peripheral erythrocytes gave low average incidences, 0.6 per 1000 in the exposed group and 0.1 per 1000 in the control, but statistically significant ($p < 0.01$) differences were found in the frequency distribution between the control and exposed groups.
Belyaev et al. [47]	Human blood lymphocytes	Chromatin condensation and 53BP1 foci	Decrease in background levels of 53BP1 foci and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation.
Busljeta et al. [48]	Rat hematopoietic tissues	MN	Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood (days 8 and 15). Concurrently, anuclear cells and erythropoietic precursor cells were decreased ($p < 0.05$) in the bone marrow on day 15, but micronucleated cells' (MNCs) frequency was increased.
d'Ambrosio et al. [49]	Human blood lymphocytes	MN	The micronucleus frequency was not affected by CW exposure; however, a statistically significant micronucleus effect was found following exposure to phase modulated field.
Diem et al. [23]	Human cultured fibroblasts and rat granulosa cells	Alkaline and neutral comet assay	The intermittent exposure showed a stronger effect in the comet assay than continuous exposure.
Ferreira et al. [50]	Rat hematopoietic tissues exposed during embryogenesis	MN	The irradiated group showed a significant increase in MN occurrence.
Fucic et al. [15]	Human blood lymphocytes	MN	X-rays and microwaves were preferentially clastogens while vinyl chloride monomer showed aneugenic activity as well. Microwaves possess some mutagenic characteristics typical of chemical mutagens.
Gadhia et al. [51]	Human blood lymphocytes	Chromosomal aberrations and SCE	There was a significant increase ($p < 0.05$) in dicentric chromosomes among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic. Synergistic action with MMC, SCEs showed a significant increase among mobile users.
Gandhi and Singh [7]	Human blood lymphocytes and buccal mucosa cells	Chromosomal aberrations and MN	Increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.
Gandhi, 2005 [52]	Human blood lymphocytes	Comet assay, *in vivo* capillary MN	Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The *in vivo* capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated cells.
Garaj-Vrhovac et al [53]	Human blood lymphocytes	Chromosomal aberrations and MN	In all experimental conditions, the frequency of all types of chromosomal aberrations was significantly higher than in the control samples. In the irradiated samples the presence of dicentric and ring chromosomes was established. The incidence of micronuclei was also higher in the exposed samples.
Garaj-Vrhovac et al. [54]	Chinese hamster cells V79	DNA synthesis by [3H]thymidine uptake, and chromosomal aberrations	In comparison with the control samples there was a higher frequency of specific chromosome lesions in cells that had been irradiated.
Garaj-Vrhovac et al. [55]	Chinese hamster cells V79	Chromosomal aberrations and MN	Significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells. The presence of micronuclei in irradiated cells confirmed the changes that had occurred in chromosome structure.
Garaj-Vrhovac et al. [56]	Human blood lymphocytes	MN	Increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.
Haider et al. [57]	*Tradescantia* flower buds	MN	The results at all exposure sites except one were statistically significant.
Koyama et al. [12]	CHO-K1 cells	MN + kinetochore determination	RF at SAR of 78 W/kg and higher form MN with a particular increase of kinetochore-positive MN and potentiate MN formation induced by bleomycine treatment.
Lai et al. [58]	Rat brain cells	Comet assay	RFR exposure significantly increased DNA double strand breaks in brain cells of the rat, and the effect was partially blocked by treatment with naltrexone.
Lai and Singh [59]	Rat brain cells	Alkaline comet assay	No effects immediately after 2 h of exposure to pulsed microwaves, whereas a dose rate-dependent increase in DNA single strand breaks was found in brain cells of rats at 4 h post-exposure with CW and pulsed waves.

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Lai and Singh [60]	Rat brain cells	Comet assay	Significantly higher levels of DNA single and double strand breaks. Exposure to 'noise' alone did not significantly affect the levels, however, simultaneous 'noise' exposure blocked microwave-induced increases in DNA strand breaks.
Lai and Singh [61]	Rat brain cells	Comet assay	An increase in DNA strand breaks was observed after exposure to either the pulsed or continuous-wave radiation, no significant difference was observed between the effects of the two forms of radiation.
Lai and Singh [35]	Rat brain cells	Comet assay	Treatment immediately before and after RFR exposure with either melatonin or *N*-tert-butyl-alpha-phenylnitrone (PBN) blocks induction of DSB by RFR. It is hypothesized that free radicals are involved in RFR-induced DNA damage in the brain cells of rats.
Lixia et al. [62]	Human lens epithelial cells	Comet assay and BudR incorporation	No DNA breaks at 1 and 2 W/kg but increase 0 and 30 min after exposure to 3 W/kg. Exposure at 2 and 3 W/kg for 2 h significantly increased HsP 70 protein but not mRNA expression.
Maes et al. [63]	Human blood lymphocytes	Chromosome aberrations	Some cytogenetic damage was obtained *in vitro* when blood samples were very close to the antenna. The questionable *in vivo* results (six maintenance workers) are not considered here.
Maes et al. [64]	Human blood lymphocytes	Chromosomal aberrations, SCE, and MN	Marked increase in the frequency of chromosome aberrations (including dicentric chromosomes and acentric fragments) and 19 micronuclei. On the other hand, the microwave exposure did not influence the cell kinetics nor the sister-chromatid-exchange (SCE) frequency.
Markova et al. [65]	Human blood lymphocytes	p53 binding protein and γH2AX foci	MWs from GSM mobile telephones affect chromatin conformation and 53BP1/gamma-H2AX foci similar to heat shock.
Mashevich et al. [66]	Human blood lymphocytes	Chromosomal aberrations	A linear increase in chromosome 17 aneuploidy was observed as a function of the SAR value.
Mazor et al. [9]	Human blood lymphocytes	Aneuploidy rate of Chr. # 1, 10, 11, 17 determined by interphase FISH	Increased levels of aneuploidy in chromosomes 1 and 10 at higher SAR, while for chromosomes 11 and 17 the increases were observed only for the lower SAR.
Nikolova et al. [67]	Mouse nestin-positive neural progenitor cells	Transcript of specific genes and proteins, proliferation, apoptosis, DNA DSB	Down-regulation of neural-specific Nurr1and up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double strand breaks.
Paulraj and Behari [68]	Rat brain cells	Comet assay	Statistically significant ($p < 0.001$) increase in DNA single strand breaks in brain cells of rat.
Pavicic and Trosic [13]	V79 cells	Alteration of microtubule proteins	The microtubule structure altered after 3 h of irritation.
Phillips et al. [69]	Molt-4 T-lymphoblastoid cells	Comet assay	DNA damage decreased by (1) exposure to the iDEN signal (2.4 μW/g for 2 h or 21 h), (2) exposure to the TDMA signal (2.6 μW/g for 2 h and 21 h), (3) exposure to the TDMA signal (26 μW/g for 2 h), exposure to the iDEN signal (24 μW/g for 2 h) and 21 h significantly increased DNA damage.
Sarimov et al. [70]	Human blood lymphocytes	Chromatin condensation by anomalous viscosity	Analysis of pooled data from all donors showed statistically significant effect of 1-h exposure to MW. Effects differ at various GSM frequencies and vary between donors.
Sarkar et al. [71]	Mouse testis and brain cells	Restriction pattern after HinfI treatment	As compared to control animals, band patterns in exposed animals were found to be distinctly altered in the range of 7–8 kb which was also substantiated by densitometric analysis.
Schwarz et al. [33]	Human cultured fibroblasts and lymphocytes	Alkaline comet assay and MN	UMTS exposure increased the CTF and induced centromere-negative micronuclei in human cultured fibroblasts in a dose- and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin.
Sykes et al. [22]	pKZ1 mice	lacZ transgene inversion	No difference between the control and treated groups in the 1- and 5-day exposure groups, but a reduction in inversions below the spontaneous frequency in the 25-day exposure group. This suggests that RF radiation can lead to a perturbation in recombination frequency.
Tice et al. [72]	Human blood lymphocytes	Alkaline comet assay and MN	Exposure for either 3 or 24 h with the unmodulated signal did not induce a significant increase in DNA DSB or MN in lymphocytes. However, with the modulated signal there was a significant and reproducible increase in the frequency of micronucleated lymphocytes.
Tkalec et al. [14]	*Allium cepa* seeds	Germination, mitotic index, mitotic abnormalities	Increased mitotic aberrations in root meristematic cells of *A. cepa*. Effects were markedly dependent on the field frequencies applied as well as on field strength and modulation. Findings also indicate that mitotic effects of RF-EMF could be due to impairment of the mitotic spindle.
Trosic et al. [73]	Rat hematopoietic tissues	MN and polychromatic erythrocytes (PCEs)	The incidence of micronuclei/1000 PCEs in peripheral blood was significantly increased ($p < 0.05$) in the subgroup exposed to fro/MW radiation after eight irradiation treatments of 2 h each in comparison with the sham-exposed control group.

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Table 1 (*Continued*)

Reference	Biological system	Genotoxic endpoint	Results and comments
Trosic et al. [74]	Rat hematopoietic tissues	MN and polychromatic erythrocytes	In polychromatic erythrocytes significant differences ($p < 0.05$) for experimental days 8 and 15. The frequency of micronucleated PCEs was also significantly increased on experimental day 15 ($p < 0.05$).
Trosic and Busljeta [75]	Rat hematopoietic tissues and peripheral blood	MN and polychromatic erythrocytes	BMPCEs were increased on days 8 and 15, and PBPCEs were elevated on days 2 and 8 ($p < 0.05$).
Vijayalaxmi et al. [76]	C3H/HeJ cancer prone mice, peripheral blood and bone marrow	MN	No observed RF effects. A correction was published, stating that there was actually a significant MN increase in peripheral blood and bone marrow cells after chronic exposure to RF [Vijayalaxmi, M.R. Frei, S.J. Dusch, V. Guel, M.L. Meltz, J.R. Jauchem, Radiat. Res. 149 (3) (1998) 308].
Wu et al. [39]	Human epithelial lens cells	Comet assay and intracellular ROS	RF at 4 W/kg for 24 h significantly increased intracellular ROS and DNA damage. Both can be blocked completely by electromagnetic noise.
Yadav and Sharma [8]	Exfoliated buccal cells	MN in buccal cells	In exposed subjects 9.84 ± 0.745 micronucleated cells and 10.72 ± 0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75 ± 0.774 MNC and 4.00 ± 0.808 TMN in controls. Correlation between 0–1, 1–2, 2–3 and 3–4 years of exposure and the frequency of MNC and TMN.
Yao et al. [40]	Human lens epithelial cells	Alkaline comet assay, gamma-H2AX foci, ROS level	SAR of 3 and 4 W/kg induced significant DNA damage in the comet assay, while no statistical difference in double strand breaks was found by γH2AX foci. Electromagnetic noise could block RF-induced ROS formation and DNA damage.
Yao et al. [41]	Human lens epithelial cells	Alkaline comet assay, γH2AX foci, ROS level	DNA damage was significantly increased by comet assay at 3 and 4 W/kg, whereas double strand breaks by γH2AX foci were significantly increased only at 4 W/kg. Significantly increased ROS levels were detected in the 3 and 4 W/kg groups.
Zhang et al. [77]	Chinese hamster lung cells (CHL)	γH2AX foci	Increased percentage of γH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 h ($37.9 \pm 8.6\%$) or 2-acetylaminofluorene exposure ($50.9 \pm 9.4\%$). However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 h ($31.8 \pm 8.7\%$).
Zotti-Martelli et al. [78]	Human blood lymphocytes	MN	Both spontaneous and induced MN frequencies varied in a highly significant way among donors ($p < 0.009$) and between experiments ($p < 0.002$), and a statistically significant increase of MN, although rather low, was observed dependent on exposure time ($p = 0.0004$) and applied power density ($p = 0.0166$).
Zotti-Martelli et al. [79]	Human blood lymphocytes	MN	The results showed for both radiation frequencies an induction of micronuclei as compared to the control cultures at a power density of 30 mW/cm^2 and after an exposure of 30 and 60 min.

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethansulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ1O), ethylmethansulfonate (EMS), chromosomal aberration analysis (CA), micronucleus assay (MN), reactive oxygen species (ROS), and fluorescence *in vitro* hybridization (FISH).

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Table 2
Publications which do not report RF-EMF related genotoxic effects.

Reference	Biological system	Genotoxic endpoint	Results and comments
Antonopouloset al. [80]	Human blood lymphocytes	SCE	No increase in SCE or cell cycle progression found.
Belyaev et al. [81]	Rat brain, spleen, and thymus	Comet assay	GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells.
Bisht et al. [82]	Mouse C3H 10T cells	MN	CDMA (3.2 or 4.8 W/kg) or FDMA (3.2 or 5.1 W/kg) RF-EMF radiation for 3, 8, 16 or 24 h did not result in a significant increase either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.
Chang et al. [83]	*Escherichia coli* tester strain	Bacterial mutagenicity (Ames test)	No mutagenic or co-mutagenic effect with 4-NQ1O.
Ciaravino et al. [84]	CHO cells	SCE	Radiofrequency electromagnetic radiation (RF-EMF) did not change the number of SCEs that were induced by adriamycin.
Garson et al. [85]	Human blood lymphocytes	CA	No RF-EMF effect observed.
Gorlitz et al. [86]	B6C3F1 mice lymphocytes, erythrocytes, and keratinocytes	MN	No visible effect.
Gos et al. [87]	*Saccharomyces cerevisiae*	Mutation rates	No effects in fluctuation tests on forward mutation rates at CAN1, on the frequency of petite formation, on rates of intra-chromosomal deletion formation, or on rates of intra-genic recombination in the absence or presence of MMS.
Hook et al. [88]	Molt-4 T lymphoblastoid cells	Comet assay	No RF-EMF effects observed.
Juutilainen et al. [89]	Female CBA/S mice and K2 female transgenic mice	MN in erythrocytes	No effect on MN frequency.
Kerbacher et al. [90]	CHO cells	CA	No alteration was observed in the extent of chromosome aberrations induced by either simultaneous fro radiation exposure or convection heating to equivalent temperatures.
Komatsubara et al. [91]	Mouse m5S cells	CA	No effect on CA; temperature increase up to 41 °C at 100 W/kg.
Koyama et al. [92]	CHO cells	MN	No MN increase in cells exposed to HFEMF at a SAR of lower than 50 W/kg, while those at SARs of 100 and 200 W/kg were significantly higher when compared with the sham-exposed controls (temperature effect).
Lagroye et al. [93]	Rat brain cells	Alkaline comet assay	No observed effect.
Lagroye et al. [94]	C3H 10T1/2 cells	Comet assay, DNA–protein crosslinks	No observed effect.
Li et al. [95]	Murine C3H 10T cells	Comet assay	No observed effect.
Maes et al. [96]	Human blood lymphocytes	CA, SCE	Combined exposure of RF-EMF and to MMC and X-rays. Overall, no indication was found of a mutagenic, and/or co-mutagenic/synergistic effect.
Maes et al. [97]	Human blood lymphocytes	CA, SCE	Combined treatments with X-rays or MMC did not provide any indication of a synergistic action between the RF-EMF fields and X-rays or MMC.
Maes et al. [98]	Human blood lymphocytes	CA, SCE, Comet assay	The alkaline comet assay, SCE, and CA tests revealed no evidence of RF-EMF-induced genetic effects. No cooperative action was found between the electromagnetic field exposure and MMC using either the comet assay or SCE test.
Malyapa et al. [99]	Rat brain cells	Comet assay	No significant differences observed.
Malyapa et al. [100]	U87MG and C3H 10T1/2 cells	Comet assay	No significant differences observed.
Malyapa et al. [101]	U87MG and C3H 10T1/2 cells	Comet assay	No significant differences observed.
McNamee et al. [102]	Human blood lymphocytes	Comet assay and MN	No significant differences observed.
McNamee et al. [103]	Human blood lymphocytes	Comet assay and MN	No significant differences observed.
McNamee et al. [104]	Human blood lymphocytes	Comet assay	No significant differences observed.
Meltz et al. [105]	L5178Y mouse leukemic cells	Mutation in TK locus	No effect of RF-EMF alone or in the induced mutant frequency due to the simultaneous exposure to RF-EMF and proclaim, as compared with the proflavin exposures alone.
Ono et al. [106]	lacZ-transgenic mice	Mutations at the lac gene in spleen, liver, brain and testis	Mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were similar to those observed in non-exposed mice.

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Table 2 (*Continued*)

Reference	Biological system	Genotoxic endpoint	Results and comments
Roti Roti et al. [107]	C3H 10T1/2 cells	Transformed foci	No statistically significant differences observed.
Sakuma et al. [108]	Human glioblastoma A172 cells and fetal lung fibroblasts	DNA strand breaks (comet assay?)	No statistically significant differences.
Scarfi et al. [109]	Human blood lymphocytes	MN	No statistically significant differences observed.
Speit et al. [24]	Human cultured fibroblasts	Comet assay and MN	No statistically significant differences observed.
Stronati et al. [110]	Human blood lymphocytes	Comet assay, CA, SCE, MN	By comparison with appropriate sham-exposed and control samples, no effect of RF-EMF alone could be found for any of the assay endpoints. In addition RF-EMF did not modify any measured effects of the X-radiation.
Takahashi et al. [111]	Big Blue mice brain tissues	lacZ transgene inversion	No statistically significant differences observed.
Verschaeve et al. [112]	Rat brain and liver tissues, erythrocytes	MN (erythrocytes) and comet assay	No genotoxic effect of RF-EMF alone. Co-exposures to MX and RF-EMF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only.
Vijayalaxmi et al. [113]	Human blood lymphocytes	CA and MN	No observed RF-EMF effects.
Vijayalaxmi et al. [114]	Human blood lymphocytes	CA and MN	No observed RF-EMF effects.
Vijayalaxmi et al. [115]	Human blood lymphocytes	Comet assay	No observed RF-EMF effects.
Vijayalaxmi et al. [116]	Human blood lymphocytes	CA, MN	No observed RF-EMF effects.
Vijayalaxmi et al. [117]	Rat hematopoietic tissues and erythrocytes	MN	No observed RF-EMF effects.
Vijayalaxmi et al. [118]	Rat whole body and head only exposures. BM erythrocytes	MN	No observed RF-EMF effects.
Vijayalaxmi et al. [119]	CF-1 male mice, peripheral blood and bone marrow	MN	No observed RF-EMF effects.
Zeni et al. [120]	Human blood lymphocytes	Comet assay, CA, SCE	No observed RF-EMF effects.
Zeni et al. [121]	Human blood lymphocytes	MN	No observed RF-EMF effects.

Abbreviations: Chromosomal aberration analysis (CA), methotrexat (MX), mitomycin C (MMC), 4-nitroqinoline-1-oxide (4-NQ1O), methylmethansulfonate (MMS), code division multiple access (CDMA), frequency division multiple access (FDMA), and time division multiple access (TDMA).

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Table 3
Publications which report synergistic RF-EMF effects in combination with other genotoxicants.

Reference	Genotoxic agents	Biological system	Genotoxic endpoint	Results and comments
Baohong et al. [122]	MMC, BLM, MMS, 4-NQ1O	Human blood lymphocytes	Alkaline comet assay	1.8 GHz RFR (SAR, 3 W/kg) for 2 h did not induce DSB, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4-NQ1O. The synergistic DNA damage effects with BLM or MMS were not obvious.
Baohong et al. [123]	254 nm UVC	Human blood lymphocytes	Alkaline comet assay	RF exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5 and 4 h incubation respectively.
Kim et al. [124]	Cyclophosphamide, 4-NQ1O, EMS	L5178Y mouse lymphoma cells (comet assay) and CHL cells (CA)	Alkaline comet assay and CA	No direct cytogenetic effect of RF alone or in combination with cyclophosphamide or 4-NQ1O was found in the CA test and in the comet assay. However, RF had a potentiating effect in combination with cyclophosphamide or 4-NQ1O.
Maes et al. [125]	MMC	Human blood lymphocytes	SCE	Synergistic effect was observed with MMC.
Maes et al. [126]	MMC	Human blood lymphocytes	CA, SCE, comet assay	The combined exposure of the cells to the radiofrequency fields followed by their cultivation in the presence of mitomycin C revealed a very weak effect when compared to cells exposed to mitomycin C alone.
Manti et al. [11]	Previous 4 Gy X-ray radiation	Human blood lymphocytes	Chromosome aberration by FISH	No significant variations due to the UMTS exposure in the fraction of aberrant cells, but frequency of exchanges per cell in X-ray irradiated cells was significantly increased by UMTS at 2 W/kg.
Wang et al. [127]	254 nm UVC	Human blood lymphocytes	Comet assay	RF did not induce DNA damage but reduced or enhanced DNA damage by UVC at 1.5 or 4.0 h respectively.
Wang et al. [128]	MMC, BLM, MMS, 4-NQ1O	Human blood lymphocytes	Comet assay	RF did not induce DNA damage but enhanced DNA damage induced by MMC and 4-NQ1O.
Zhang et al. [129]	MMC	Human blood lymphocytes	Comet assay, micronucleus assay	No RF-induced DNA and chromosome damage, but increased MMC DNA damage by RF in comet assay.

Abbreviations: Mitomycin C (MMC), bleomycin (BLM), methylmethansulfonate (MMS), 4-nitroquinoline-1-oxide (4-NQ1O), ethylmethansulfonate (EMS), chromosomal aberration analysis (CA), fluorescence *in vitro* hybridization (FISH).

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fragments of chromosomes or from lagged chromosomes secondary to mitotic non-disjunction, the latter being detected by indirect immunofluorescence using kinetochore antibodies. Kinetochore-positive MN arise by epigenetic mechanisms (disturbances of the spindle apparatus). Kinetochore-negative MN arise from acentric chromosomal fragments. This is an important distinction, but has been performed in a few RF-EMF studies only, of which only one [12] reports an increase of kinetochore-positive MN albeit after a high SAR $\geq$ 78 W/kg. Two studies describe RF-EMF-induced disturbances of the spindle apparatus [13,14], and one reports an aneugenic RF-EMF effect on the basis of the size distribution of MN [15]. Of a total of 39 studies using the micronucleus assay 22 are MN-positive, and 17 MN-negative.

SCEs are analysed in metaphase chromosomes after two rounds of replication in the presence of 5-bromodeoxyuridine (BUDR). SCEs, which are induced during the S-phase of the cell cycle, represent an exchange between homologous chromatids, an event which by itself is genetically neutral. Nevertheless it is considered to reflect a recombinational repair of DNA double strand breaks (DSB), and may therefore serve as an indicator of genotoxic stress. Of 10 studies using SCE a GT(+) effect was reported in one only, 8 were negative, and one study reports RF-induced enhancement of genotoxicity by mitomycin C.

3. DNA fragmentation

The comet assay, also known as a "Single Cell Gel electrophoresis assay" (SCG), and the detection of gamma-H2AX foci are the most frequently used techniques to study RF-EMF-induced DNA strand breaks. The comet assay uses interphase nuclear DNA, which is unwinded under alkaline conditions and subsequently subjected to an electric field. Here DNA fragments migrate towards the anode, thereby forming a comet-like tail [16,17]. The alkaline comet assay detects DNA single strand as well as double strand breaks, but is not applicable in the presence of DNA crosslinking agents [18]. These breaks may occur not only by toxic influences but also by transcriptional and repair processes and by alkali-sensitive sites. Therefore this frequently used and very sensitive assay has a poor specificity. Of 41 studies using the comet assay 15 report comet-positive and 19 comet-negative results after RF-EMF exposure. RF-EMF enhancement of comet assay effects caused by other genotoxic agents is described in 7 studies.

Out of a multitude of DNA damage checkpoint proteins two have been used to detect DBS: H2AX, a member of the nuclear histone family [19], and P53 binding protein (53BP1). Both are rapidly phosphorylated only minutes after DNA damage and are then gathered in the vicinity of DNA double strand breaks. Here they form foci which can be visualized by indirect immunofluorescence [20,21]. These foci represent an initial and specific step in the repair process of exogenously induced DNA double strand breaks. It is important to realize, however, that repair processes of DSB are quantified, not DSB themselves. The method has been employed in 4 studies, predominantly using the yH2AX foci test. In all instances GT(+) effects have been detected.

DNA alterations have also been analysed by the anomalous viscosity time dependency test (AVTD, 1 GT(+) study), detecting conformational changes, and by quantitative PCR (QPCR, 1 GT(+) study) detecting structural changes in the DNA.

4. Gene mutations

In this category 6 studies have been performed using 4 different endpoints: (1) Altered restriction fragments (1 GT(+) study), (2) lacZ inversion in transgenic mice. This method has been used in 3 studies which all failed to detect an increased rate of inversions, but one found a reduced rate as compared to unexposed controls [22], which is interpreted as a RF-EMF-induced reduction of recombination repair. (3) Mutation at the thymidine kinase (TK) locus (1 negative study). (4) Bacterial his^- revertants (Ames test, 1 negative study).

5. Discussion

The large number of contradictory results among the 101 published studies on a genotoxic action of RF-EMF is tangling. Nevertheless patterns can be perceived. GT(+) as well as GT(−) findings have been reported at a standard absorption ratio (SAR) below 0.05 up to 100 W/kg and an exposure of 15 min and 48 h *in vitro*, and between hours and years *in vivo*. The outcome of studies was nearly independent from RF frequencies between 300 and 7700 MHz and the type of RF signal, either continuous wave (CW) or pulse-modulated (PM). GT(+) was obtained in 15 CW and 26 PM exposures, GT(−) in 14 CW and 27 PM exposures (some studies did not indicate the type of signal used). Contradictory results have been obtained even when two experienced groups performed the same experiments using the same cells and identical exposure conditions [23,24]. This may reflect a general problem of genotoxic studies being dependent on a multitude of factors which are difficult to control [25]. Some of the studies exploited here have shortcomings with respect to incompletely described or unreliable exposure conditions and/or an inadequate experimental design. Even a considerable publication bias in favour of negative results has been suspected (www.microwavenews.com/RR.html, 2006) [26].

The proportion of GT(+) effects is much higher *in vivo* (23/40) than *in vitro* (29/77). (Since some studies have been performed on more than one biological system, the total number of GT(+) and GT(−) effects exceeds the total number of published studies.) Considering all genotoxic endpoints applied, the frequently used parameters chromosome analysis (9/21 GT(+)), comet assay (15/41 GT(+)), and sister-chromatid-exchange (1/10 GT(+)) showed the highest

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proportion of negative results, while the micronucleus assay yielded more positive than negative results (22/39 GT(+)). Since the SCE test which was negative in nearly all cases is known to be rather insensitive to radiomimetic (clastogenic) agents it can be speculated, that a clastogenic mechanism is involved in RF-EMF genotoxic action.

Epigenetic influences may also contribute to genotoxicity as demonstrated by RF-EMF-induced chromosomal non-disjunction and disturbances of the mitotic spindle. This is in agreement with the higher proportion of 22/39 GT(+) findings among studies using the micronucleus assay as compared to those using CA, because some of the micronuclei may represent lagged chromosomes. Epigenetic mechanisms may also be effective after a combined exposure to RF-EMF and various physical or chemical mutagens (Table 4). RF-EMF preferentially enhanced the genotoxic effect of 4-NQ1O (4/4), MMC (4/8), UVC (2/2), and cyclophosphamide (2/2). No synergistic effect was obtained using MMS and EMS (3/3), BLM (2/2), and adriamycine (2/2). Only one out of 3 studies reported a synergistic effect with X-rays.

Cells and tissues of different origin exhibit a clearly variable sensitivity for genotoxic RF-EMF effects (Table 4). This has also been observed with extremely low frequency (ELF)-EMF [27] and may be dependent on genetic differences [28]. GT(+) effects of RF-EMF were reported predominantly in the following biological systems: human lens epithelial cells (4/4), human buccal mucosa cells (2/2), rodent brain tissues (8/13), and rat hemopoietic tissues (5/7). GT(−) results have been obtained with mouse permanent cell lines (7/7) and permanent lymphoblastoid cells of various origin (7/7). This is in a striking analogy to RF-EMF-induced reduction of ornithine decarboxylase activity being detected in primary but not in secondary neural cells [29].

6. Proposed mechanisms of RF-EMF genotoxicity

Cells are unusually sensitive to electromagnetic fields [30]. Weak fields may accelerate electron transfer and thereby destabilize the H-bond of cellular macromolecules. This could explain the stimulation of transcription and protein expression, which has been observed after RF-EMF exposure [31,32]. However, the energy of weak EM fields is not sufficient directly to break a chemical bond in DNA. Therefore it can be concluded, that genotoxic effects are mediated by indirect mechanisms as microthermal processes, generation of oxygen radicals (ROS), or a disturbance of DNA-repair processes.

6.1. Thermal effects

An increase of temperature in the culture medium of RF-EMF exposed cells has been observed at very high SAR levels only [12]. The vast majority of GT(+) studies were conducted at SAR < 2.0 not leading to a detectable increase of temperature in the culture medium. Moreover, similar or larger effects have been observed at a 5′ on/10′ off intermittent exposure [23,33], a result that contradicts a

Table 4
Distribution RF-EMF effects in 101 published studies.

Biological system	RF-EMF effects		Synergistic effects	
	Positive	Negative	Positive	Negative
In vitro (all cells and tissues)	29	39	9	11
Human blood lymphocytes	18	23	8	4
Human lens epithelial cells	4			
Human cultured fibroblasts	2	2		
Human glioblastoma cells		3		
Human lymphoblastoid cells		2		
Mouse permanent cell lines		6		1
Mouse lymphoblastoid cells		1	1	1
Chinese hamster cells (CHO, V79)	4	2		3
E. coli		1		2
Yeast		1		
Rat granulosa cells	1			
In vivo (all species and tissues)	23	17	0	1
Human blood lymphocytes	4	2		
Human buccal mucosa cells	2			
Mouse sperm	1			
Mouse brain tissues	2			
Mouse polychromatic erythrocytes		4		
Rat brain tissues	6	4		1
Rat hemopoietic tissues	5	2		
Rat spleen, liver		2		
lacZ-transgenic mice		3		
Plants	2			
Cattle polychromatic erythrocytes	1			

Since several published studies have used more than 1 biological system the total of negative and positive effects exceeds the number of 101 publications.

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simple temperature-based mechanism of the observed genotoxic action. However, experimental results with microwave absorption at colloidal interfaces have demonstrated that the electric absorption of microwaves between 10 and 4000 MHz goes through a maximum with the size of bride droplets >100 and <10,000 nm, and depends on the type of ions and their concentrations [34]. This local absorption of microwaves may therefore lead to a considerable local heating in living cells during low energy microwave exposure.

6.2. Oxygen radicals

There is evidence that RF-EMF may stimulate the formation of reactive oxygen species in exposed cells *in vivo* [35–37] and *in vitro* [38–41]. Free oxygen radicals may form base adducts in DNA, the most important lesion being 8-OHdG, and oxidize also other cellular components, such as lipids leaving behind reactive species, that in turn can couple to DNA bases [42]. The first step in the generation of ROS by microwaves is mediated in the plasma membrane by NADH oxidase [43]. Subsequently ROS activates matrix metalloproteases (MMP), thereby initiating intracellular signalling cascades. It is interesting to note that these processes start within 5 min of radiation and at a very low field intensity of 0.005 W/cm^2. Moreover, higher effects have been obtained by intermittent radiation, when cells were left unirradiated for 10 min. This is in agreement with *in vitro* genotoxicity studies using the comet assay [23,33].

6.3. Alteration of DNA-repair processes

A considerable proportion of studies have investigated the consequences of a combined exposure to RF-EMF and various chemical or physical mutagens. 8/12 studies using human blood lymphocytes have demonstrated that RF-EMF enhanced the genotoxic action of other agents, preferentially of UV, MMC, or 4-NQ1O (an UV-mimetic agent). Since in all these experiments microwave exposure failed to induce detectable genotoxic effect by itself, an interference with DNA-repair mechanisms has been postulated, however, there is no direct experimental proof yet. An alteration of recombinational repair has also been proposed by Sykes et al. [22] as an explanation of the reduced rate of inversions in lacZ-transgenic mice after RF-EMF treatment.

An influence of microwave exposure on DNA-repair processes has long been proposed for power frequency electromagnetic fields [35]. A recent epidemiological investigation into the frequency of polymorphisms of DNA-repair genes in children with acute leukemia living in the vicinity of power line transformers [44] emphasizes the significance DNA-repair impairment for an EMF related increase of this malignancy. There was a significant gene–environment interaction (COR = 4.31) between the electromagnetic field intensities and a less active genetic variant of XRCC1, a crucial enzyme in base excision repair.

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Please cite this article in press as: H.W. Ruediger, Genotoxic effects of radiofrequency electromagnetic fields, Pathophysiology (2009), doi:10.1016/j.pathophys.2008.11.004

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Please cite this article in press as: H.W. Ruediger, Genotoxic effects of radiofrequency electromagnetic fields, Pathophysiology (2009), doi:10.1016/j.pathophys.2008.11.004

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Pathophysiology xxx (2009) xxx–xxx

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Epidemiological evidence for an association between use of wireless phones and tumor diseases

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Received 7 November 2008; accepted 30 January 2009

Abstract

During recent years there has been increasing public concern on potential cancer risks from microwave emissions from wireless phones. We evaluated the scientific evidence for long-term mobile phone use and the association with certain tumors in case–control studies, mostly from the Hardell group in Sweden and the Interphone study group. Regarding brain tumors the meta-analysis yielded for glioma odds ratio (OR) = 1.0, 95% confidence interval (CI) = 0.9–1.1. OR increased to 1.3, 95% CI = 1.1–1.6 with 10 year latency period, with highest risk for ipsilateral exposure (same side as the tumor localisation), OR = 1.9, 95% CI = 1.4–2.4, lower for contralateral exposure (opposite side) OR = 1.2, 95% CI = 0.9–1.7. Regarding acoustic neuroma OR = 1.0, 95% CI = 0.8–1.1 was calculated increasing to OR = 1.3, 95% CI = 0.97–1.9 with 10 year latency period. For ipsilateral exposure OR = 1.6, 95% CI = 1.1–2.4, and for contralateral exposure OR = 1.2, 95% CI = 0.8–1.9 were found. Regarding meningioma no consistent pattern of an increased risk was found. Concerning age, highest risk was found in the age group <20 years at time of first use of wireless phones in the studies from the Hardell group. For salivary gland tumors, non-Hodgkin lymphoma and testicular cancer no consistent pattern of an association with use of wireless phones was found. One study on uveal melanoma yielded for probable/certain mobile phone use OR = 4.2, 95% CI = 1.2–14.5. One study on intratemporal facial nerve tumor was not possible to evaluate due to methodological shortcomings. In summary our review yielded a consistent pattern of an increased risk for glioma and acoustic neuroma after >10 year mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term exposure and needs to be revised.

Keywords: Brain tumors; Glioma; Acoustic neuroma; Meningioma; Cellular phones; Cordless phones

1. Introduction

During the last decade there has been a rapid development of wireless technology and along with that an increased use of wireless telephone communication in the world. Most persons use mobile phones and cordless phones. Additionally most populations are exposed to radiofrequency/microwave (RF) radiation emissions from wireless devices such as cellular antennas and towers, broadcast transmission towers, voice and data transmission for cell phones, pagers and personal digital assistants and other sources of RF radiation.

Concerns of health risks have been raised, primarily an increased risk for brain tumors, since the brain is the near field target organ for microwave exposure during mobile phone calls. Especially the ipsilateral brain (same side as the mobile phone has been used) is exposed, whereas the contralateral side (opposite side to the mobile phone) is much less exposed [1]. Thus, for risk analysis it is of vital importance to have information on the localisation of the tumor in the brain and which side of the head that has been predominantly used during phone calls.

Since Sweden was one of the first countries in the world to adopt this wireless technology a brief history is given in the following. First, analogue phones (NMT; Nordic Mobile Telephone System) were introduced on the market in the early 1980s using both 450 and 900 Megahertz (MHz) carrier waves. NMT 450 was used in Sweden since 1981 but closed down in December 31, 2007, whereas NMT 900 operated during 1986–2000.

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doi:10.1016/j.pathophys.2009.01.003

Table 1
Odds ratios (ORs) and 95% confidence intervals (CIs) from 11 case–control studies on glioma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

Author, year of publication, country, reference number	No. of cases	No. of controls	OR	95% CI
Inskip et al., 2001, USA [23]	201	358	1.0	0.7–1.4
Auvinen et al., 2002, Finland [24]	Not given	Not given	1.5	1.0–2.4
Lönn et al., 2005, Sweden [25][a]	214	399	0.8	0.6–1.0
Christensen et al., 2005, low-grade glioma, Denmark [26][a]	47	90	1.1	0.6–2.0
Christensen et al., 2005, high-grade glioma, Denmark [26][a]	59	155	0.6	0.4–0.9
Hepworth et al., 2006, UK [27][a]	508	898	0.9	0.8–1.1
Schüz et al., 2006, Germany [28]	138	283	1.0	0.7–1.3
Hardell et al., 2006, Sweden [12], all glioma	346	900	1.4	1.1–1.7
Low-grade glioma	65	900	1.4	0.9–2.3
High-grade glioma	281	900	1.4	1.1–1.8
Lahkola et al., 2006, Denmark, Norway, Finland, Sweden, UK [29]	867	1 853	0.8	0.7–0.9
Hours et al., 2007, France [30]	59	54	1.2	0.7–2.1
Klaeboe et al., 2007, Norway [31][a]	161	227	0.6	0.4–0.9
Takebayashi et al., 2008, Japan [17]	56	106	1.2	0.6–2.4
Meta-analysis	>1667[b]	>3554[b]	1.0	0.9–1.1

[a] Not included in meta-analysis because already part of pooled data in Lahkola et al., 2006 [29].
[b] Total number could not be calculated since numbers were not presented in one publication [24].

The digital system (GSM; Global System for Mobile Communication) using dual band, 900 and 1800 MHz, started to operate in 1991 and now dominates the market. The third generation of mobile phones, 3G or UMTS (Universal Mobile Telecommunication System), using 1900 MHz RF broad band transmission has been introduced worldwide since a few years, in Sweden since 2003.

Desktop cordless phones have been used in Sweden since 1988, first analogue 800–900 MHz RF fields, but since early 1990s the digital 1900 MHz DECT (Digital Enhanced Cordless Telecommunications) system is used. In our studies on tumor risk associated with use of wireless phones, we have also assessed use of cordless phones. However, most other research groups have not published such data at all, or only in a scanty way, so exposure to RF from DECT is not further discussed here. Instead the reader is referred to our previous publications on this issue [2–13].

The initial studies on brain tumor risk had too short latency periods to give a meaningful interpretation. However, during recent years studies have been published that enable evaluation of ≥10-years latency period risk, although still mostly based on low numbers [14,15]. A ≥10-years latency period seems to be a reasonable minimum period to indicate long-term carcinogenic risks from exposure to RF fields during use of mobile or cordless phones.

Table 2
Odds ratios (ORs) and 95% confidence intervals (CIs) from six case–control studies on glioma including meta-analysis of the studies using ≥10 year latency period. Numbers of exposed cases and controls are given.

Study	Total			Ipsilateral			Contralateral		
Author, year of publication, country, latency, reference number	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Lönn et al., 2005, Sweden, ≥10 years [25][a]	25/38	0.9	0.5–1.5	15/18	1.6	0.8–3.4	11/25	0.7	0.3–1.5
Christensen et al., 2005, Denmark, low-grade glioma, ≥10 years [26][a]	6/9	1.6	0.4–6.1	–	–	–	–	–	–
Christensen et al., 2005, Denmark, high-grade glioma, ≥10 years [26][a]	8/22	0.5	0.2–1.3	–	–	–	–	–	–
Hepworth et al., 2006, UK, ≥10 years [27][a]	66/112	0.9	0.6–1.3	Not given	1.6	0.9–2.8	Not given	0.8	0.4–1.4
Schüz et al., 2006, Germany, ≥10 years [28]	12/11	2.2	0.9–5.1	–	–	–	–	–	–
Hardell et al., 2006, Sweden, >10 years [12], all glioma	78/99	2.7	1.8–3.9	41/28	4.4	2.5–7.6	26/29	2.8	1.5–5.1
Low-grade glioma	7/99	1.5	0.6–3.8	2/28	1.2	0.3–5.8	4/29	2.1	0.6–7.6
High-grade glioma	71/99	3.1	2.0–4.6	39/28	5.4	3.0–9.6	22/29	3.1	1.6–5.9
Lahkola et al., 2006, Denmark, Norway, Finland, Sweden, UK, ≥10 years [29]	143/220	0.95	0.7–1.2	77/117	1.4	1.01–1.9	67/121	1.0	0.7–1.4
Meta-analysis	233/330	1.3	1.1–1.6	118/145	1.9	1.4–2.4	93/150	1.2	0.9–1.7

[a] Not included in meta-analysis because already part of pooled data in Lahkola et al., 2006 [29].

Please cite this article in press as: L. Hardell, et al., Epidemiological evidence for an association between use of wireless phones and tumor diseases, Pathophysiology (2009), doi:10.1016/j.pathophys.2009.01.003

Table 3
Odds ratios (ORs) and 95% confidence intervals (CIs) from nine case–control studies on acoustic neuroma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

Author, year of publication, country, reference number	No. of cases	No. of controls	OR	95% CI
Inskip et al., 2001, USA [23]	40	358	0.8	0.5–1.4
Lönn et al., 2004, Sweden [32][a]	89	356	1.0	0.6–1.5
Christensen et al., 2004, Denmark [33][a]	45	97	0.9	0.5–1.6
Schoemaker et al., 2005, Denmark, Finland, Sweden, Norway, Scotland, England [34]	360	1934	0.9	0.7–1.1
Hardell et al., 2006, Sweden [11]	130	900	1.7	1.2–2.3
Takebayashi et al., 2006, Japan [35]	51	192	0.7	0.4–1.2
Klaeboe et al., 2007, Norway [31][a]	22	227	0.5	0.2–1.0
Schlehofer et al., 2007, Germany [36]	29	74	0.7	0.4–1.2
Hours et al., 2007, France [30]	58	123	0.9	0.5–1.6
Meta-analysis	668	3581	1.0	0.8–1.1

[a] Not included in meta-analysis because already part of pooled data in Schoemaker et al., 2005 [34].

Long-term exposure to RF fields from mobile phones and brain tumor risk is of importance to evaluate, not the least since the use of cellular phones is globally widespread with high prevalence among almost all age groups in the population. In the following we discuss mobile phone use and the association with brain tumors, but also other tumor types that have been studied. Recently, we published a detailed review of studies on brain tumors [14] followed by meta-analyses of published studies regarding glioma, acoustic neuroma and meningioma [15]. We have now recalculated these results with the addition of two new recently published articles from the Interphone study group [16,17]. Studies from individual countries were only included in the meta-analyses if they were not also included in the joint publications for several countries. For odds ratio (OR) and 95% confidence interval (CI) we used fixed effects model as in the recent publication by Kundi [18]. The analyses were done using Stata/SE 10 (Stata/SE 10 for Windows; StataCorp., College Station, TX).

One case–control study was excluded since no separate data were presented for glioma, acoustic neuroma or meningioma [19], and another since no overall data on acoustic neuroma were published, only for some time periods without results for ≥10 year latency period [20].

Due to several methodological limitations a Danish cohort study on "mobile phone subscribers" [21] is not possible to include in the meta-analysis, and the same methodological shortcomings prevail in the published updated cohort [22]. In the following only a short overview of the results for brain tumors is given, since we have discussed these issues in more detail elsewhere [14,15]. The other tumor types that have been studied are salivary gland tumors, non-Hodgkin lymphoma (NHL), testicular cancer, eye melanoma and facial nerve tumor.

2. Glioma

Glioma is a malignant type of brain tumor and comprises about 60% of all central nervous system tumors. The highly malignant glioblastoma multiform, with poor survival, is included in this group.

Eleven case–control studies present results for glioma [12,17,23–31]. Of these eight [17,25–31] were part of the Interphone study and four of these [25–27,31] were included in a pooled-analysis with additional data for Finland [29]. The results are presented in Table 1. Overall no decreased

Table 4
Odds ratios (ORs) and 95% confidence intervals (CIs) from four case–control studies on acoustic neuroma including meta-analysis of the studies using ≥10 year latency period. Numbers of exposed cases and controls are given.

Study	Total			Ipsilateral			Contralateral		
Author, year of publication, country, latency, reference number	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Lönn et al., 2004, Sweden, ≥10 years [32][a]	14/29	1.8	0.8–4.3	12/15	3.9	1.6–9.5	4/17	0.8	0.2–2.9
Christensen et al., 2004, Denmark, ≥10 years [33][a]	2/15	0.2	0.04–1.1	–	–	–	–	–	–
Schoemaker et al., 2005, Denmark, Finland, Sweden, Norway, Scotland, England, ≥10 years [34]	47/212	1.0	0.7–1.5	31/124	1.3	0.8–2.0	20/105	1.0	0.6–1.7
Hardell et al., 2006, Sweden, >10 years [11]	20/99	2.9	1.6–5.5	10/28	3.5	1.5–7.8	6/29	2.4	0.9–6.3
Meta-analysis	67/311	1.3	0.97–1.9	41/152	1.6	1.1–2.4	26/134	1.2	0.8–1.9

[a] Not included in meta-analysis because already part of pooled data in Schoemaker et al., 2005 [34].

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or increased risk was found for glioma in the meta-analysis; OR = 1.0, 95% CI = 0.9–1.1.

Results for 10 year latency period are presented in Table 2. Six studies [12,25–29] gave such information and three [25–27] of these were also part of the publication by Lahkola et al. [29]. The meta-analysis yielded significantly increased risk for glioma with OR = 1.3, 95% CI = 1.1–1.6 increasing to OR = 1.9, 95% CI = 1.4–2.4 for ipsilateral exposure. The latter results were based on 118 exposed cases and 145 exposed controls. Regarding contralateral exposure to microwaves from mobile phones a lower risk was calculated, OR = 1.2, 95% CI = 0.9–1.7 (n = 93 cases, 150 controls). It should be noted that in the study by Takebayashi et al. [17] analyses of maximum microwave energy absorbed at the location of the tumor gave OR = 1.6, 95% CI = 0.6–4.2 related to the highest quartile of cumulative phone time weighted by maxSAR and OR = 5.8, 95% CI = 0.96–36 for subjects with cumulative maxSAR-hour of ≥10 W/kg-h.

3. Acoustic neuroma

These tumors are benign and do not undergo malignant transformation. They tend to be encapsulated and grow in relation to the auditory and vestibular portions of nerve VIII. They are slow growing tumors initially in the auditory canal, but gradually grow out into the cerebellopontine angle, where they come into contact with vital brain stem centers.

Nine case–control studies have been published [11,23, 30–36], see Table 3. Seven [30–36] were part of the Interphone study and three [31–33] were included in the publication by Schoemaker et al. [34]. Analysis of the total material yielded OR = 1.0, 95% CI = 0.8–1.1 increasing to 1.3, 95% CI = 0.97–1.9 using 10 year latency period, Table 4. For ipsilateral exposure OR increased further to 1.6, 95% CI = 1.1–2.4, whereas contralateral exposure gave a non-significantly increased risk, OR = 1.2, 95% CI = 0.8–1.9.

4. Meningioma

Meningioma arises from the pia or archnoid, which are the covering layers of the central nervous system. The majority are benign tumors that are encapsulated and well-demarched from surrounding tissue.

Regarding meningioma results have been published from nine case–control studies, Table 5 [11,16,17,23,25,26, 28,30,31]. Of these, seven [16,17,25,26,28,30,31] were part of the Interphone studies. The Lahkola et al. study [16] included three separately published Interphone studies [25,26,31]. The meta-analysis in Table 5 gave a significantly reduced OR = 0.9, 95% CI = 0.8–0.9. These results were mainly caused by the findings in the Interphone study [16] with the largest numbers of cases and controls yielding OR = 0.8, 95% CI = 0.7–0.9 in that study.

Using 10 year latency period OR was close to unity and somewhat increased for ipsilateral exposure, OR = 1.3, 95% CI = 0.9–1.8, Table 6. Regarding contralateral exposure OR was non-significantly decreased to 0.8, 95% CI = 0.5–1.3. The results for laterality were based on only two studies [11,16].

5. Brain tumor risk in different age groups

We grouped cases and controls according to age when they started to use a mobile or a cordless phone [11,12]. Consistently we found the highest risk for those with first use <20 years age. Thus, for malignant brain tumors OR = 2.7, 95% CI = 1.3–6.0 was calculated for mobile phones and OR = 2.1, 95% CI = 0.97–4.6 for cordless phones. The corresponding results for benign brain tumors were OR = 2.5, 95% CI = 1.1–5.9 and OR = 0.6, 95% CI = 0.2–1.9, respectively. Previously, we published results for diagnosis of brain tumor in different age groups [37] and found highest OR = 5.9, 95% CI = 0.6–55 for ipsilateral use of analogue phones in the youngest age group 20–29 years at the time of diagnosis. Using a >5 years latency period increased the risk further.

6. Brain tumor risk for use of mobile phone in urban and rural areas

There is a difference in output power of digital mobile phones between urban and rural areas. Adaptive power control (APC) regulates power depending on the quality of the transmission. In rural areas with on average longer distance to the base station the output power level is higher than in urban areas with dense population and shorter distance to the base stations. We studied the risk for brain tumors in urban versus rural living from the data in our study with cases diagnosed January 1, 1997 to June 30, 2000 [38]. Regarding digital phones OR = 1.4, 95% CI = 0.98–2.0 was obtained for living in rural areas increasing to OR = 3.2, 95% CI = 1.2–8.4 with >5 years latency period. The corresponding results for living in urban areas were OR = 0.9, 95% CI = 0.8–1.2 and OR = 0.9, 95% CI = 0.6–1.4, respectively.

7. Salivary gland tumors

The salivary glands, especially the parotid gland, are targets for near-field microwave exposure during calls with wireless phones. A Finnish study reported OR = 1.3, 95% CI = 0.4–4.7 for those who had ever had a mobile phone subscription [24].

Results from three case–control studies have been published, one from Sweden, one from the Nordic countries and one from Israel. During the same period as our studies on brain tumors we performed a study on salivary gland tumors [39]. Our study included the whole Swedish pop-

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Table 5
Odds ratios (ORs) and 95% confidence intervals (CIs) from nine case–control studies on meningioma including meta-analysis of the studies. Numbers of exposed cases and controls are given.

Author, year of publication, country, reference number	No. of cases	No. of controls	OR	95% CI
Inskip et al., 2001 (USA) [23]	67	358	0.8	0.5–1.2
Lönn et al., 2005 (Sweden) [25][a]	118	399	0.7	0.5–0.9
Christensen et al., 2005 (Denmark) [26][a]	67	133	0.8	0.5–1.3
Schüz et al., 2006 (Germany) [28]	104	234	0.8	0.6–1.1
Hardell et al., 2006 (Sweden) [11]	347	900	1.1	0.9–1.3
Klaeboe et al., 2007 (Norway) [31][a]	96	227	0.8	0.5–1.1
Hours et al., 2007 (France) [30]	71	80	0.7	0.4–1.3
Lahkola et al., 2008 (Denmark, Norway, Finland, Sweden, UK) [16]	573	1696	0.8	0.7–0.9
Takebayashi et al., 2008, Japan [17]	55	118	0.7	0.4–1.2
Meta-analysis	1217	3386	0.9	0.8–0.9

[a] Not included in meta-analysis because already part of pooled data in Lahkola et al., 2008 [16].

ulation. Cases were recruited by using the regional cancer registries, and most had a malignant disease. They were diagnosed during 1994–2000, but with some variation for the different medical regions in Sweden. Population based controls were used as reference group. The questionnaire was answered by 267 (91%) of the cases and 750 (92%) of the controls. Of the cases 245 had a cancer diagnosis. Overall no association was found; analogue phones yielded OR = 0.9, 95% CI = 0.6–1.4, digital OR = 1.0, 95% CI = 0.7–1.5 and cordless phones OR = 1.0, 95% CI = 0.7–1.4. No effect of tumor induction period was found, although regarding >10 year latency period only 6 cases had used an analogue phone, OR = 0.7, 95% CI = 0.3–1.7, whereas no case had used a digital or cordless phone with that latency period. The results did not change significantly for ipsilateral or contralateral tumors.

The Nordic part of the Interphone case–control study of an association between use of mobile phones and parotid gland tumors was published in 2006 [40]. Detailed information about mobile phone use was obtained from 60 (85%) cases with malignant tumor, 112 (88%) with benign tumor and 681 (70%) controls. Regular mobile phone use gave OR = 0.7, 95% CI = 0.4–1.3 for malignant tumors and OR = 0.9, 95% CI = 0.5–1.5 for benign parotid gland tumors. For ipsilateral mobile phone use a latency period of ≥10 year yielded OR 0.7, 95% CI = 0.1–5.7 for malignant tumors ($n = 1$) and OR = 2.6, 95% CI = 0.9–7.9 for benign tumors ($n = 6$). Contralateral use was reported by one case with benign tumor and no case with malignant tumor in the same latency group.

As part of the Interphone study results on parotid gland tumor were reported from Israel [41]. It included 402 benign and 58 malignant incident cases, total 460 (87%) of 531 eligible for the time period 2001–2003. Population based matched controls were used, in total 1266 (66%) out of 1920 eligible subjects. Thirteen cases had a latency period of ≥10 year, which gave OR = 0.9, 95% CI = 0.4–1.8. No significantly increased risk was found for duration of use; ≥10 year yielded OR = 1.0, 95% CI = 0.5–2.1. However, for cumulative number of calls >5479 OR = 1.6, 95% CI = 1.1–2.2 was found for ipsilateral and both ears used equally, whereas contralateral use gave OR = 0.8, 95% CI = 0.5–1.2. Similarly, cumulative call time >266.3 h yielded OR = 1.5, 95% CI = 1.1–2.1; contralateral use gave OR = 0.8, 95% CI = 0.6–1.3.

In the meta-analysis using 10 year latency period no overall increased risk was found, OR = 0.8, 95% CI = 0.5–1.4, but for ipsilateral use it increased to OR = 1.7, 95% CI = 0.96–2.9, whereas contralateral use gave OR = 0.4, 95% CI = 0.2–1.2, Table 7.

Table 6
Odds ratios (ORs) and 95% confidence intervals (CIs) from five case–control studies on meningioma including meta-analysis of the studies using ≥10 year latency period. Numbers of exposed cases and controls are given.

Study	Total			Ipsilateral			Contralateral		
Author, year of publication, country, latency, reference number	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Lönn et al., 2005, Sweden, ≥10 years [25][a]	12/36	0.9	0.4–1.9	5/18	1.3	0.5–3.9	3/23	0.5	0.1–1.7
Christensen et al., 2005, Denmark, ≥10 years [26][a]	6/8	1.0	0.3–3.2	–	–	–	–	–	–
Schüz et al., 2006, Germany, ≥10 years [28]	5/9	1.1	0.4–3.4	–	–	–	–	–	–
Hardell et al., 2006, Sweden, >10 years [11]	38/99	1.5	0.98–2.4	15/28	2.0	0.98–3.9	12/29	1.6	0.7–3.3
Lahkola et al., 2008 (Denmark, Norway, Finland, Sweden, UK) [16]	73/212	0.9	0.7–1.3	33/113	1.1	0.7–1.7	24/117	0.6	0.4–1.03
Meta-analysis	116/320	1.1	0.8–1.4	48/141	1.3	0.9–1.8	36/146	0.8	0.5–1.3

[a] Not included in meta-analysis because already part of pooled data in Lahkola et al., 2008 [16].

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Table 7
Odds ratios (ORs) and 95% confidence intervals (CIs) from three case–control studies on salivary gland tumors including meta-analysis of the studies using ≥10 year latency period.

Study	Total			Ipsilateral			Contralateral		
Author, year of publication, country, latency, reference number	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI	No. of cases/controls	OR	95% CI
Hardell et al., 2004, Sweden, >10 years [39]	6/35	0.7	0.3–1.7	5/13	1.5	0.5–4.2	1/15	0.3	0.03–2.1
Lönn et al., 2006, malignant, Sweden, ≥10 years [40]	2/36	0.4	0.1–2.6	1/23	0.7	0.1–5.7	0/19	–[a]	–[a]
Lönn et al., 2006, benign, Sweden, ≥10 years [40]	7/15	1.4	0.5–3.9	6/9	2.6	0.9–7.9	1/9	0.3	0.0–2.3
Sadetzki et al., 2007, Israel, ≥10 years [41]	13/26	0.9	0.4–1.8	10/16	1.6	0.7–3.7	3/10	0.6	0.2–2.3
Meta-analysis	28/112	0.8	0.5–1.4	22/61	1.7	0.96–2.9	5/34	0.4	0.2–1.2

[a] Not included in meta-analysis because OR could not be estimated.

8. Non-Hodgkin lymphoma

The incidence of NHL increased since the 1960s in Sweden as well as in many western countries with reliable cancer registries. This trend has levelled off since the 1990s, and decreasing exposure to environmental contaminants such as PCBs and dioxins, and also certain pesticides has been postulated to be one explanation [42,43]. As part of a large case–control study on NHL, mainly on exposure to pesticides [44], also questions on the use of wireless phones were included. The study covered the time period December 1, 1999 to April 30, 2002. The questionnaire was answered by 910 (91%) cases and 1016 (92% controls). The majority of the cases had B-cell NHL and we did not find any association with use of wireless phones [45]. Regarding T-cell NHL ($n=53$) we observed somewhat increased risks; use of analogue phone gave OR = 1.5, 95% CI = 0.6–3.7, digital phone OR = 1.9, 95% CI = 0.8–4.8 and cordless phone OR = 2.5, 95% CI = 1.1–5.6. For certain subtypes of T-cell NHL, the cutaneous and leukemia types, the risks increased further for analogue phone to OR = 3.4, 95% CI = 0.8–15, digital phone to OR = 6.1, 95% CI = 1.3–30, and cordless phone to OR = 5.5, 95% CI = 1.3–24. These results were, however, based on low numbers.

A study from USA included 551 NHL cases and 462 frequency matched controls [46]. Among regular mobile phone users NHL risk was not significantly associated with minutes per week, duration, cumulative lifetime or years of first use. However, total time >8 years gave OR = 1.6, 95% CI = 0.7–3.8. The risk increased with number of years, and was significant for the not specified group of NHL after ≥6 years use yielding OR = 3.2, 95% CI = 1.2–8.4.

9. Testicular cancer

An increasing incidence of testicular cancer has been noted in most western countries during the recent decades. It is the most common cancer type in young men and is not regarded to be an occupational disease. Cryptorchidism is an established risk factors, but also perinatal exposure to persistent organic pollutants with hormone activity has been suggested to be another risk factor [47,48]. There has been concern in the population that use of mobile phones might be a risk factor for testicular dysfunction. We performed a case–control study mainly on the use of PVC plastics as risk factor for testicular cancer [49], and included in the questionnaire also questions on the use of wireless phones. The results were based on answers from 542 (92%) cases with seminoma, 346 (89%) with non-seminoma and 870 (89%) controls [50]. Overall no association was found [50]. Only 13 cases with seminoma had used an analogue phone >10 years yielding OR = 2.1, 95% CI = 0.8–5.1 and one case with non-seminoma; OR = 0.3, 95% CI = 0.04–2.6. No case had used a digital or cordless phone with latency period >10 years. OR did not increase with cumulative use in hours for the different phone types. Regarding use of mobile phone in the stand by mode border line significance was found for seminoma, OR = 1.3, 95% CI = 1.03–1.7, but not for non-seminoma; OR = 0.9, 95% CI = 0.7–1.3. For different localisations during stand by, highest risk was found for seminoma for keeping the phone in ipsilateral trousers pocket, OR = 1.8, 95% CI = 0.97–3.4 whereas contralateral pocket gave OR = 1.0, 95% CI = 0.5–2.0.

10. Malignant melanoma of the eye

Stang et al. [51] conducted a hospital- and population-based case–control study of uveal melanoma and occupational exposures to different sources of radiofrequency radiation. A total of 118 cases with uveal melanoma and 475 controls were included. Exposure to RF-transmitting devices was rated as (a) no RF exposure, (b) possible exposure to mobile phones, or (c) probable/certain exposure to mobile phones. An elevated risk for exposure to RF-transmitting devices was reported. Exposure to radio sets gave OR = 3.0, 95% CI = 1.4–6.3 and probable/certain exposure to mobile

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phones OR = 4.2, 95% CI = 1.2–14.5. The authors concluded that several methodologic limitations prevented their results from providing clear evidence on the hypothesized association.

The study was commented among others Johansen et al. [52]. In their cohort of mobile phone subscribers in Denmark no support for an association between mobile phones and ocular melanoma was found. However, as discussed elsewhere [14,15,18,55], there are several methodological limitations in the Danish cohort [21,22] that hamper the interpretation of their findings.

The paper by Stang et al. [51] has also been commented by Inskip [53] in an editorial, the main point being that missing from the paper is any consideration of occupational or recreational exposure to UV radiation.

11. Intratemporal facial nerve tumor

So far only one investigation has studied the risk of intratemporal facial nerve (IFN) tumor and the use of mobile phone [54]. A case–control approach was used with 18 patients with IFN tumors matched with controls ($n = 192$) treated for other diseases, 51 patients treated for acoustic neuroma, 72 treated for rhinosinusitis, and 69 for dysphonia and gastroesophageal reflux. Risk of facial nerve tumorigenesis was compared by extent of mobile phone use. The OR of developing an IFN tumor was 0.6, 95% CI = 0.2–1.9 with any handheld mobile phone use and OR = 0.4, 95% CI = 0.1–2.1 for regular mobile phone use. However, they concluded that the short duration of use precludes definite exclusion as a risk for IFN tumor development. Certainly the cases were too few for a sound epidemiological study and it was not correct to include patients with acoustic neuroma in the reference group.

12. Discussion

A review on use of mobile phones and the association with brain tumors included all case–control studies that we have identified in the peer-review literature. Most studies have published data with rather short latency period and limited information on long-term users.

No other studies than from the Hardell group has published comprehensive results for use of cordless phones (DECT) [2–15]. As we have discussed in our publications it is pertinent to include also such use in this type of studies. Cordless phones are an important source of exposure to microwaves and they are usually used for a longer time period on daily basis as compared to mobile phones. Thus, to exclude such use, as was done in e.g. the Interphone studies, could lead to an underestimation of the risk for brain tumors from use of wireless phones.

We have discussed shortcomings in the Interphone studies in detail elsewhere [55]. Regarding glioma the Swedish Interphone study reported 23 ORs in Table 2 in that publication [25] and 22 of these were <1.0 and one OR = 1.0. For meningioma all 23 ORs were <1.0, six even significantly so. These results indicate a systematic bias in the study unless use of mobile phones prevents glioma and meningioma, which is biologically unlikely. It should be noted that several of the overall ORs also in other Interphone studies were <1.0, some even significantly so. As an example, in the Danish Interphone study on glioma [26] all 17 ORs for high-grade glioma were <1.0, four significantly decreased. Also other Interphone studies reported ORs significantly <1.0, that is a protective effect or rather systematic bias in the studies [16,29,31].

Use of cellular telephones was mostly assessed by personal interviews in the Interphone studies. It is not described how these personal interviews were organized, a tremendous task considering that vast parts of Sweden from north to south had to be covered. In the sparsely populated and extended area in northern Sweden personal interviews must have meant lots of long distance traveling and imposed additional stress on the interviewers. No information was given in the articles on how or if this methodological problem was solved, for example were controls only included from more densely populated areas.

The interviews in the Interphone study were extensive and computer aided. It is likely that such an interview creates a stressful situation for a patient with a recent brain tumor diagnosis and operation. These patients, especially under pressure with a newly diagnosed brain tumor and possible surgery, often have difficulties remembering past exposures and inevitably have problems with concentration and may have problems with other cognitive shortcomings. In the Danish part of the Interphone study it was concluded that the patients scored significantly lower than controls due to recalling words (aphasia), problems with writing and drawing due to paralysis [26]. According to our experience a better option would have been to start with a mailed questionnaire, that can be answered by the patient during a period of more well-being, if necessary this can be complemented by a telephone interview. After surgery it is easier to answer a questionnaire at home, also with the possibility to check phone bills to verify the use. This procedure has the additional advantage that it can be accomplished without disclosure during the data collection, whether a person is a case or a control. Certainly, knowing if it was a case or a control that was interviewed in the Interphone study may have introduced observational bias.

It has been argued that recall bias might be introduced in case–control studies on cancer patients, since the patients would be more prone to find a cause for their disease than the controls. However, the contrary is often the situation since patients do not want to blame themselves for their disease. In one article we presented data on the patients own assumptions of causes of their brain tumor [5]. Of 1429 cases only two expressed concern about mobile phones and no about cordless

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phones. Interestingly, cases with a previous cancer diagnosis reported lower frequency for use of wireless phones than those with no previous cancer. No interviewer bias could be demonstrated when exposure data in the questionnaire were compared before and after phone interviews [5].

The diagnosis of tumor type as well as grading is based on histopathology. X-ray investigation or MR alone is insufficient. Of the 371 cases with glioma in the Swedish Interphone study [25] histopathology examination of the tumor was available for 328 (88%) cases, and for 225 (82%) of the meningioma cases. Thus, it is possible that cases without histology confirmation of the diagnosis may have had another type of brain tumor or even brain metastases. Such misclassifications inevitably bias the result towards unity. It is remarkable that 345 glioma cases were stratified according to grade I–IV, although histopathology was available only for 328 cases. In our studies on brain tumors we have histopathology verification of all of the diagnoses. Also, the total number of included cases [25] is not completely consistent with those reported to the Swedish Cancer Registry as we have discussed elsewhere [55]. The study included cases from neurosurgery, oncology and neurology clinics as well as regional cancer registries in the study areas.

Among the controls in the glioma and meningioma study 282 (29%) refused to participate [25]. Among some of these non-responders a short interview was made and only 34% reported regular use of a cellular telephone compared with 59% of the responders. If this discrepancy extends to the total group of non-responders the true percentage of mobile phone users in controls would be approximately 52%. Hence this figure would be lower than in glioma (58% exposed) and acoustic neuroma cases (60%). Only for meningioma with 43% exposed cases a lower percentage was reported, however, considering the sex ratio (women:men) for meningioma of about 2:1 a lower percentage of mobile phone users has to be expected due to the lower rate of users among women. It should be noted that a similar procedure in another Interphone study yielded similar results regarding mobile phone use among responders and non-responders [17].

It was discussed in a medical dissertation [56] that: 'Our Swedish study, that includes a large number of long-term mobile phone users, does not support the few previously reported positive findings, and does not indicate any risk increases neither for short-term or long-term exposures.' Considering the methodological shortcomings and that in contrast to the cited assertion of 'a large number of long-term users' the study subjects included only 25 glioma and 12 meningioma cases with long-term use, its conclusion seems to be going a long way beyond what can be scientifically defended.

It might be mentioned that this area of research seems to be controversial *per se* with unfounded statements [57], easily rebutted [58] and not supported by evolving scientific evidence [59]. Statements on no risk for brain tumors based on short-time use of mobile phones [60] might be considered in a larger context [61].

We included in our studies use of mobile or cordless phone 'any time' in the exposed group and made dose-response calculations based on number of hours of cumulative use. The unexposed group included also subjects with use of wireless phones with ≤1-year latency period. On the contrary, mobile phone use in the Interphone studies was defined as 'regular use' on average once per week during at least 6 months, less than that was regarded as unexposed including also all use within <1 year before diagnosis. This definition of 'regular use' seems to have been arbitrary chosen and might have created both observational and recall bias in the interpretation of such a definition.

Use of cordless phones was not assessed or not clearly presented in the Interphone studies, e.g. [25,28]. We found a consistent pattern of an association between cordless phones and glioma and acoustic neuroma [11,12]. It has been shown that the GSM phones have a median power in the same order of magnitude as cordless phones [62]. Moreover, cordless phones are usually used for longer calls than mobile phones [11,12]. Including subjects using cordless phones in the "unexposed" group in studies on this issue, as for example in the Interphone investigations, would thus underestimate the risk and bias OR against unity.

The case participation was good in our studies, 88% for cases with benign brain tumors, 90% for malignant brain tumor cases and 89% for the controls. On the contrary case participation varied from 37% to 93% and control participation from 42% to 75% in the Interphone studies. Obviously low participation rates for cases and controls might give selection bias and influence the results in the Interphone studies.

Methodological issues in the Interphone studies have been discussed elsewhere [14,15,18,55,63–65]. It was concluded that the actual use of mobile phones was underestimated in light users and overestimated in heavy users. Random recall bias could lead to large underestimation in the risk of brain tumors associated with mobile phone use. It was further suggested that selection bias in the Interphone study resulted in under selection of unexposed controls. Refusal to participate was related to less prevalent use of mobile phones, and this could result in a downward bias in estimates of the disease risk associated with mobile phone use. As discussed by Kundi [18] there was also interview lag time between cases and controls in the Interphone studies that might have been a source of bias due to the fast increase of mobile phone use during the study period. This could have resulted in underestimation of risk.

For salivary gland tumors the results were based on three case-control studies. In the 10 year latency period the meta-analysis gave an almost significantly increased risk for ipsilateral use of mobile phones, and a non-significantly decreased risk for contralateral use. These results were based on few cases. Regarding NHL and testicular cancer some subgroup analysis yielded increased risks, but these results were based on low numbers. Use of mobile phone increased the risk significantly for melanoma of the eye. The study on intratemporal facial nerve tumors is not informative since

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it was based on few cases and included acoustic neuroma patients in the control group. It is concluded that all studies were hampered by low numbers of long-term users and need to be replicated for firm evidence of an association between use of mobile phones and these tumor types.

In summary our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after >10 years mobile phone latency. Our studies showed also an association with use of cordless phones, an issue that has not been studied at all in most investigations or only rudimentary in two studies. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term exposure and needs to be revised.

Acknowledgements

Supported by grants from Cancer-och Allergifonden, Cancerhjälpen and Örebro University Hospital Cancer Fund. Contribution by co-workers in the various publications is acknowledged.

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